FECAL MICROBIOTA TRANSPLANTATION FOR INDUCTION OF REMISSION IN MILD TO MODERATE ACTIVE CROHN'S DISEASE

A THESIS SUBMITTED TO BABA FARID UNIVERSITY OF

HEALTH SCIENCES, FARIDKOT (PUNJAB)

IN THE PARTIAL FULFILLMENT

FOR THE AWARD OF DEGREE OF

DOCTORATE OF MEDICINE

IN

GASTROENTEROLOGY

2024

Submitted By

DR. SHIVAM BANSAL

DEPARTMENT OF GASTROENTEROLOGY

DAYANAND MEDICAL COLLEGE AND HOSPITAL

LUDHIANA, PUNJAB

DECLARATION BY THE CANDIDATE

I hereby declare that the thesis entitled **"FECAL MICROBIOTA TRANSPLANTATION FOR INDUCTION OF REMISSION IN MILD TO MODERATE ACTIVE CROHN'S DISEASE"** submitted towards partial fulfillment of the requirements for the award of **D.M. Gastroenterology** degree to BFUHS is a compilation of original work carried out by me under the supervision of **Dr. Ajit Sood,** Professor and Head, Department of Gastroenterology and **Dr. Ramit Mahajan**, Professor, Department of Gastroenterology. No part of this thesis has formed the basis for the award of any degree previously.

_____

DR. SHIVAM BANSAL
Department of Gastroenterology
Dayanand Medical College & Hospital,
Ludhiana, Punjab

Date:

Place:

CERTIFICATE OF SUPERVISORS

This is to certify that the work incorporated in this thesis entitled **"FECAL MICROBIOTA TRANSPLANTATION FOR INDUCTION OF REMISSION IN MILD TO MODERATE ACTIVE CROHN'S DISEASE"** has been carried out by **Dr. Shivam Bansal** under our direct supervision. The data incorporated in this thesis is original and genuine and has been carried out by the candidate himself.

Signature of Supervisor

Dr. Ajit Sood
Professor & Head
Department of Gastroenterology
DMCH, Ludhiana

Signature of Co-Supervisor

Dr. Ramit Mahajan
Professor
Department of Gastroenterology
DMCH, Ludhiana

Date:

ENDORSEMENT BY THE HEAD OF THE INSTITUTE

This is to certify that the thesis entitled **"FECAL MICROBIOTA TRANSPLANTATION FOR INDUCTION OF REMISSION IN MILD TO MODERATE ACTIVE CROHN'S DISEASE"** is a bonafide research work done by **Dr. Shivam Bansal** under the supervision of **Dr. Ajit Sood**, Professor and Head, Department of Gastroenterology and **Dr. Ramit Mahajan**, Professor, Department of Gastroenterology in this institution.

Principal

Dr. G S Wander
Principal
DMC&H, Ludhiana

Date:

COPYRIGHT

Declaration by the Candidate

I hereby declare that the Baba Farid University of Heath Sciences, Faridkot, shall have the rights to preserve & use this thesis in print or electronic format for academic/study purpose by the research scholar/students.

Signature

Dr. Shivam Bansal
Department of Gastroenterology
Dayanand Medical College & Hospital,
Ludhiana, Punjab

Date:

© Baba Farid University of Health Sciences, Faridkot

Dedicated

to

my family

&

Teachers

ACKNOWLEDGEMENT

I am deeply humbled to have completed this significant milestone in my academic journey, and I am compelled to express my heartfelt gratitude to the individuals who have supported me throughout this endeavor.

First and foremost, I praise God the Almighty, merciful and passionate for his showers of blessings, unparalleled grace and granting me the capability to proceed successfully and in good health. I express my sincere gratitude and sense of indebtedness to my teacher and supervisor, ***Dr. Ajit Sood****, Professor & Head, Department of Gastroenterology, Dayanand Medical College and Hospital, Ludhiana, whose extraordinary wisdom, expertise, and mentorship have been the guiding light of my research journey. Dr. Sood's exceptional leadership and mentorship have not only shaped the direction of this work but have also inspired me to strive for excellence in my academic pursuits. His guidance has been instrumental in helping me navigate the complexities of research, and his insightful feedback has been invaluable in shaping the direction of this work. I am honored to have had the privilege of working with him and I aspire to emulate his qualities of wisdom, humility, and dedication to the pursuit of knowledge.*

Words cannot express my deepest gratitude to my distinguished mentor and co-supervisor ***Dr. Ramit Mahajan****, Professor, Department of Gastroenterology, Dayanand Medical College and Hospital, Ludhiana, whose limitless efforts and keen interest helped me throughout this study. I thank him for his patience and timely advice, without which I would have floundered in this study. It is indeed my extraordinary privilege to be able to work under his expert guidance. His boundless*

expression of knowledge, guidance and constant supervision has gone a long way in ensuring the completion of this study. He provided the best encouragement, facilities and affection that helped me to overcome whatever obstacles came during my study.

I would like to express my gratitude to Dr Varun Mehta and Dr Omesh Goyal, Professors, Department of Gastroenterology, Dr Arshdeep Singh and Dr Yogesh Gupta, Associate Professors Department of Gastroenterology. Their invaluable assistance and encouragement have been significant source of motivation through phases of research.

"I would also like to thank my parents Dr. Bhushan Lal Bansal and Mrs. Madhu Bansal whose unwavering love and belief in me have been the foundation upon which I have built my academic and personal life. Their countless sacrifices, patience and encouragement have always pushed me to strive for excellence. You have been my pillars of strength, and I owe so much of who I am today to the values you instilled in me. From the very first day of my education you have been my greatest supporters, and I am forever indebted to you for all that you have done.

To my beloved wife Dr. Shibu Garg, I cannot express enough gratitude for your unrelenting support, understanding, and encouragement throughout this process. Your love, patience, and sacrifices have been my refuge during the many long nights and challenging days. You have stood by me through every step, offering not only emotional support but also practical help when needed. Thank you for always believing in me, even when I found it hard to believe in myself. Your presence

in my life has been a constant source of motivation and joy, and I share this achievement with you as much as it is my own.

I would also like to extend my heartfelt thanks to Dr. Rishu Garg, Dr. Shruti Bansal, Dr. Shubham Garg and Dr. Ankita Goyal. Your support, encouragement, and constant check-ins made this journey less daunting and more manageable. Whether it was a word of advice, a shared moment of joy, or simply knowing that you believed in me, each of you has contributed to this accomplishment in your own way.

I take this opportunity to thank my seniors Dr. Aakash Agarwal, Dr. Mayur Luthra, Dr. Gaurav Maurya, my colleagues Dr. Manisha Khubber, Dr. Mudasir Bashir Sheikh and my juniors Dr. Raghav Upneja, Dr. Tarun Jain for their constant help and encouragement throughout this journey. My gracious thanks to Dr Dharmatma Research Department DMC&H for helping me with patient counselling and enrolment and helping me during difficult times.

My sincere thanks to Gastroenterology staff Mr. Andrew Bhatti , Mr. Piyush Kumar, Mr. Abhishek Soddar and Mr. Ajay Kalda for their constant support and motivation that always proved beneficial. Heartfelt thanks to Mr. Vishal and Mr. Vikas for arranging logistics and ensuring hassle free flow of the thesis.

I acknowledge the help of all those invisible hands who are responsible for the successful outcome of this dissertation. My heartiest thanks to the nursing staff of the Department of Gastroenterology for their sincere

cooperation. Last but not the least, I would like to thank all the patients who participated in this study.

Dr. Shivam Bansal

LIST OF ABBREVIATIONS

IBD	Inflammatory Bowel Disease
FMT	Fecal Microbiota Transplantation
CDAI	Crohn's Disease Activity Index
SES-CD	Simple Endoscopic Score for Crohn Disease
5-ASA	5-Aminosalicylic Acid
AZA	Azathioprine
IFX	Infliximab
6-MP	6-Mercaptopurine
TOFA	Tofacitinib
ADA	Adalimumab
VDZ	Vedolizumab
CDI	Clostridium Difficile Infection
HBI	Harvey Bradshaw Index
CRP	C-Reactive Protein
ESR	Erythrocyte Sedimentation Rate
GI	Gastrointestinal
IV	Intravenous
CAD	Coronary Artery Disease
COAD	Chronic Obstructive Airways Disease
CVA	Cerebrovascular Accident
PCDAI	Pediatric Crohn's Disease Activity Index

RCDI	Recurrent Clostridium Difficile Infection
UC	Ulcerative Colitis
TET	Transendoscopic Enteral Tubing
CDEIS	Crohn's Disease Endoscopic Index of Severity
AE	Adverse Event
OTU	Operational Taxonomic Unit
CR	Clinical Remission
QoI	Quality of Life
IBDQ	IBD Questionnaire
ECCO	European Crohn’s and Colitis Organisation
ESGAR	European Society of Gastrointestinal and Abdominal Radiology
BMI	Body Mass Index
IQR	Inter Quartile Range
SD	Standard Deviation
IVDU	Intravenous Drug Use
PCV	Packed Cell Volume
TLC	Total Leucocyte Count
AST	Aspartate Tranaminase
ALT	Alanine Transaminase
ALP	Alkaline Phophatase

ABSTRACT

Introduction: Crohn's disease is a chronic relapsing remitting inflammatory disease of the GI tract. Conventional immunosuppressive treatment is associated with significant adverse effects. Targeting gut dysbiosis becal microbiota transplantation which plays a key role in CD pathogenesis can be safe and promising approach.

Aim of study: To evaluate efficacy of FMT in patients with mild to moderate active Crohn's disease. Primary outcomes were defined as achievement of clinical and endoscopic remission at week 20. Secondary outcomes were defined as clinical and endoscopic response at week 20.

Methods: This randomized controlled trial included patients with Crohn's disease with CDAI score between 150-449 and SES CD score ≥ 6 who were randomized in 1:1 ratio to receive either FMT or placebo via colonoscopy route at 0, 4 ,12 and 20 weeks. Clinical and endoscopic disease activity was assessed by CDAI score and SES CD score respectively at each visit. Blood samples were collected at baseline. Fecal calprotectin was performed at baseline and at week 24. Association between intervention and desired outcomes was analysed using intention to treat analysis.

Results: The study included 20 patients which were randomised to FMT group (n=11) and placebo group (n=9). Clinical remission at week 20 was seen in 7/8 patients in both FMT and placebo group (FMT :87.5%: placebo=87.5% p=1.000). Endoscopic remission at week 20 was seen in 1/8 patients allocated FMT and none of the patients given placebo. (FMT 12.5% vs placebo 0% p=0.234). Significant decrease was noted in fecal calprotectin (p=0.023) and SES CD score (p=0.002) from baseline in the FMT group but not in placebo group. No serious adverse events were noted during the study.

Conclusion: There was no significant difference in primary and secondary endpoints among two groups. Significant reduction in objective parameters of mucosal inflammation denotes potential for FMT as a safe and therapeutic option in patients with Crohn's disease. Future studies with large sample sizes and standardized protocols are required to establish FMT in therapeutic ladder of Crohn's disease.

Keywords: Crohn's disease, FMT, Clinical remission, Endoscopic remission

TABLE OF CONTENTS

S. NO.	TOPIC	PAGES
I	INTRODUCTION	1-4
II	AIMS AND OBJECTIVES	5
III	REVIEW OF LITERATURE	6-25
IV	MATERIALS AND METHODS	26-31
V	OBSERVATIONS AND RESULTS	32-74
VI	DISCUSSION	75-80
VII	SUMMARY AND CONCLUSIONS	81-82
VIII	BIBLIOGRAPHY	83-89
	APPENDICES – I - PROFORMA - MASTER CHART	
	APPENDICES – II - APPROVAL LETTER OF THESIS PLAN - THESIS PLAN	

LIST OF TABLES

Table no.	Title	Pages No.
1	Montreal Classification for adult CD	26
2	Crohn's Disease Activity Index (CDAI)	27
3	Simple endoscopic score for Crohn's disease	27
4	Baseline characteristics-1	32
5	Age at diagnosis in two groups	35
6	Disease duration in two groups	36
7	Baseline characteristics-2	37
8	Baseline BMI	39
9	Disease location distribution	41
10	Disease behaviour distribution	42
11	Association between Intervention and Baseline CDAI	43
12	Association between Intervention and Baseline HBI	44
13	Association between Intervention and Baseline SES CD	45
14	Previous treatment with intravenous steroids	46
15	Previous treatment with oral prednisolone	47
16	Previous treatment with oral budesonide	48
17	Previous treatment with oral 5-ASA	49
18	Previous treatment with azathioprine/6-MP	50
19	Previous treatment with tofacitinb	51

20	Previous treatment with adalimumab	52
21	Mean hemoglobin values at baseline	53
22	Baseline PCV values	54
23	Baseline CRP values	56
24	Baseline fecal calprotectin among two groups	57
25	Slurry retention time after first FMT	58
26	Comparison of change in slurry retention time among two groups	59
27	Comparison of change in CDAI score over study period	61
28	Comparison of change in HBI score over study period	62
29	Comparison of achievement of clinical response	63
30	Comparison of achievement of clinical remission	64
31	Comparison of change in SES CD score over study period	65
32	Percent Change in SES CD from 1st Visit to Follow-up Timepoints	66
33	Comparison of achievement of endoscopic response	67
34	Comparison of achievement of endoscopic remission	68
35	Change in Fecal calprotectin after FMT	69
36	Concomitant oral prednisolone use in two groups	70
37	Concomitant oral budesonide use in two groups	71
38	Concomitant oral 5-ASA use in two groups	72
39	Concomitant AZA use in two groups	73

40	Common side effects after FMT	74
41	Comparison of the studies done on FMT in Crohn’s disease	80

LIST OF FIGURES

Figure no.	Title	Pages No.
1	Flow diagram of the study	33
2	Age distribution	34
3	Age at diagnosis distribution	35
4	Disease duration distribution	36
5	Baseline BMI distribution	39
6	Disease location distribution	41
7	Disease behaviour distribution	42
8	Baseline CDAI among two groups	43
9	Baseline HBI among two groups	44
10	Baseline SES CD among two groups	45
11	Previous treatment with intravenous steroids	46
12	Previous treatment with oral prednisolone	47
13	Previous treatment with oral budesonide	48
14	Previous treatment with oral 5-ASA	49
15	Previous treatment with azathioprine/6-MP	50
16	Previous treatment with tofacitinib	51
17	Previous treatment with adalimumab	52
18	Hemoglobin values at baseline	53
19	Baseline PCV values among two groups	54

20	Baseline CRP values among two groups	56
21	Baseline fecal calprotectin among two groups	57
22	Slurry retention time after first FMT	58
23	Change in Slurry Retention Time Over Time	60
24	Change in CDAI score over time	61
25	Change in HBI score over time	62
26	Comparison of clinical response over time	63
27	Association between Intervention and Clinical Remission	64
28	Comparison of SES CD score over time	65
29	Comparison of difference of SES CD between the 1st Visit timepoint and the follow-up timepoints	66
30	Comparison of Endoscopic Response over time	67
31	Association between Intervention and Endoscopic Remission	68
32	Change in Fecal calprotectin after FMT	69
33	Comparison of change in concomitant oral prednisolone	70
34	Comparison of change in concomitant oral budesonide	71
35	Comparison of change in concomitant oral 5-ASA	72
36	Comparison of change in concomitant azathioprine	73
37	Side effect profile after FMT	74

KEY TO MASTER CHART

Family history	Absent-0 Present-1
Appendicectomy	Absent-0 Present-1
Addictions	Absent-0 Present-1
Comorbidities	Absent-0 Present-1
Disease location	Ileal disease-1 Colonic disease-2 Ileocolonic disease-3
Disease behaviour	Inflammatory-1 Stricturing-2
Previous treatment	Absent-0 Present-1
Concomitant treatment	Absent-0 Present-1
Adverse effects	Absent-0 Present-1

BABA FARID UNIVERSITY OF HEALTH SCIENCES
Sadiq Road, Faridkot-151203 (Pb.) INDIA
Phone No. 91-1639-256253 and Fax No. 91-1639-256234
Website: www.bfuhs.ac.in, Email: bfuhspc@gmail.com
(BFUHS – UNIVERSITY LIBRARY- Coordinator APC)

11-BFUHS (LI-PC-134)24/1226 Dated: October 14, 2024

Plagiarism Verification Certificate

Title of the thesis: **Fecal Microbiota Transplantation For Induction Of Remission In Mild To Moderate Active Crohn's Disease.**

Total Page: **82 (Eighty Two only) eFSPC (eFile Sent for Plagiarism Check)**

Researcher: **Shivam Bansal**

Supervisor: **Dr. Ajit Sood**

Research Centre: **Dayanand Medical College & Hospital, Ludhiana**

This is to report that the above thesis was scanned for similarity detection. Process and outcome is given below:

DID:	**2370463**	Software used:	**DRILLBIT**
Date:	**October 14, 2024**	Similarity Index:	**5% (Below 10 Percent)**

Total words count: **19706 (eFSPC)**

The complete report is submitted for review by the Supervisor and Co-Supervisor.

University Coordinator/
Name & Signature (Checked by)
(From University library)

The complete report of the above thesis has been reviewed by the undersigned. (Check Box)

☐ The similarity index is below accepted norms.

☐ The similarity index is above accepted norms, because of the following reasons:

1.....
2.....
3…….....
4…….....
5……

The thesis may be considered for the award of degree. (Relevant documents attached).

Research Scholar **Co-Supervisor** **Supervisor**

INTRODUCTION

Crohn's disease is a chronic relapsing-remitting inflammatory disease of the gastrointestinal tract that can involve any part from mouth to anus. The hallmark feature of Crohn's disease is the transmural inflammation of the intestinal mucosa resulting in ulcerations, fistulae, strictures, and skip lesions.[1] The pathogenesis of CD is complex but it is now accepted that it is linked to an aberrant activation of the gastro-intestinal immune system under the influence of environmental factors, infections and dysbiosis in genetically susceptible hosts.[2]

The gut microbiota plays a pivotal role in the maintenance of intestinal homeostasis and modulation of the host immune system and acts as an intestinal biological barrier against pathogens. Multifaceted microbial population is considered as a human organ with critical function.[3] Studies on intestinal gut microbiota suggest that an imbalance in the microbiota composition is linked with a dysregulated immune response.[2] This altered gut microbial composition termed dysbiosis contributes to pathogenesis of CD as suggested by recent studies.[4,5]

In healthy individuals, the gut microbiota remains relatively stable over time, whereas it is more unstable in IBD patients. The gut microbiota is influenced by variety of factors including host genotype, diet, medications, immunological status and environment. Moreover, the gut microbiota composition varies between the active and inactive stages of the disease and even in the same individual between sites of active or no inflammation. The question of dysbiosis as a cause or result of intestinal inflammation remains debated in patients with IBD.[6] Research has shown that the unaffected relatives of CD patients also had gut dysbiosis, although it differed from that observed in CD patients.[7] Furthermore, changes in gut microbiota have been associated with two major CD susceptibility genes, NOD2 and ATG18L.[8] These findings suggest that dysbiosis which is thought to be

a consequence of inflammation is driven by both genetic and environmental factors.

With the advent of non culturable techniques such as 16s RNA sequencing, faster and more complete identification of gut microbiome has been made possible. Four major phyla constitute more than 90 percent of normal gut microbiota namely Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria. Firmicutes and Bacteroidetes dominate the gut microbiota composition.[6] The Firmicutes phylum primarily comprises of Clostridium XIV and IV groups. Alterations in the gut microbiota in CD patients include reduced diversity; decreased abundance of several taxa such as Faecalibacterium, Ruminococcaceae, and Bacteroides; and increased abundance of Gammaproteobacteria and Fusobacteriaceae.[4] Research has shown that patients with Crohn disease have more significant gut dysbiosis than those with ulcerative colitis.[5,9]

Efforts have been made to explore dysbiosis as a diagnostic tool or biomarker. Lower relapse rates were seen in post operative CD patients with a gut microbiota composition similar to the healthy subjects as compared to those with dysbiosis.[10] Additionally, studies have shown that patients with less diverse gut microbiota tend to have more severe symptoms, while those with greater diversity are more likely to be in remission.[11] Ongoing research is focused on the potential use of gut microbial profiles to assess disease activity, predict outcomes, and guide treatment strategies.

Conventional treatment for CD is based on the immunosuppressive medications such as aminosalicylates, corticosteroids and thiopurines prior to escalation to biological therapies such as anti TNF agents and anti-Interleukins. Approximately 30 percent of patients show no response to these treatments. Significant adverse effects such as serious infections and lymphoma can also occur in some patients and many patients require surgery within 10 years of diagnosis.[12] Multiple

studies have been done on manipulating the gut microbiota and thereby targeting dysbiosis and intestinal inflammation. Prebiotics, probiotics and antibiotics have tried in various trials but the results of these trials have been mixed. Modulation of the gut microbiome through fecal microbiota transplantation appears as promising treatment strategy in IBD. The first FMT ever done was by Ge Hong during the 4th century in China in patients with food poisoning or severe diarrhea.[13] The first report in the medical literature was in 1958 by Eiseman and his colleagues who successfully treated patients with FMT for pseudomembranous colitis.[14] FMT has shown greater than 90% success rates in treatment for recurrent clostridium difficile infection(RCDI) in recent studies.[15,16] Due to the satisfactory effect of FMT for RCDI, FMT has been added in the treatment guidelines of RCDI in 2013 and 2014 by the American Society of Gastroenterology, the European Association of Clinical Microbiology, and European Association of Infectious Diseases.[17,18] The first case using FMT for UC patients was reported by Bennet et al in 1989.[19] Since then, multiple case reports, case series, and randomized controlled trials had been reported investigating the efficacy and safety of FMT in IBD. Studies have shown FMT as an effective treatment for induction and maintenance of remission in Ulcerative colitis patients.[20-23]

Studies on effectiveness of FMT in the Crohn's disease management is limited and the results have been mixed.[24-26] There are several factors in Crohn's disease that may contribute to less than desired response with FMT as compared with ulcerative colitis. Gut dysbiosis is more pronounced Crohn's disease patients as compared to ulcerative colitis patients.[5] Mucosal adherent bacteria are thought to play a vital role in pathogenesis of Crohn's disease which may be less amenable to alteration with fecal microbiota transplantation.[27] Also, the success of fecal microbiota transplantation likely depends on targeted delivery of donor microbiota to the diseased gut which can be challenging in small bowel Crohn's disease. The transmural and progressive nature of inflammation in Crohn's disease patients may also obligate more

intensive and prolonged fecal microbiota transplantation for treatment success.[9]

There is limited understanding of whether the transfer of a metabolically active and dynamic microbiota through FMT can influence the metabolism of Crohn's disease patients. The metabolome made up of low molecular weight byproducts of bacterial metabolism, represents the body’s overall response to conditions like disease and specific treatments. Metabolomics provides a unique approach to identifying significant metabolic changes that occur following FMT. Further studies are needed to explore metabolomic changes and to determine an ideal metabolome profile in CD patients.

The long-term outcomes of FMT have been studied and current evidence suggest that FMT is a safe therapeutic option with minimal adverse effects. Most clinical trials and systemic reviews reported minor transient adverse events after FMT such as abdominal discomfort, diarrhea, constipation, and low-grade fever. Serious adverse effects were uncommon and were often linked with the complications of endoscopy and sedation.[28,29]

Overall FMT can be a promising treatment option in patients with Crohn’s disease with few long term side effects. In this study, we conducted a randomized controlled trial to assess the efficacy of FMT in Crohn's disease patients using both clinical and endoscopic outcomes.

AIMS AND OBJECTIVES

Outcomes

Primary outcome were defined as

1. Achievement of clinical remission (defined as CDAI <150) at week 20
2. Achievement of endoscopic remission (SES CD<3) at week 20

Secondary outcomes were defined as

1. Clinical response (defined as decline in CDAI by > 100 points) at week 20
2. Endoscopic response (defined as >50% decline in SES CD) at week 20

REVIEW OF LITERATURE

In recent years, there has been an increasing interest in modifying the intestinal microbiota composition to target gut dysbiosis. Convincing data and studies are available regarding the use and efficacy of FMT in recurrent Clostridium Difficile infection and ulcerative colitis but there is limited evidence for use of FMT in Crohn's disease.

FMT involves transplanting fecal material from a healthy donor into the recipient's gastrointestinal tract. Donors undergo thorough screening with stool and serology tests and must meet specific inclusion and exclusion criteria for stool donation.[30] Clinical evidence indicates no association between donor relation and FMT outcomes, therefore both related and unrelated donors can be considered.[31] Additionally, the timing between donor screening and stool donation is crucial; the French FMT group recommends that this period should not exceed 21 days to minimize contamination risk.[32] The debate over whether fresh or frozen fecal material is preferable for FMT has persisted since the practice began. However, recent randomized clinical trials and meta-analyses have shown that frozen FMT is just as effective as fresh FMT in treating recurrent or refractory CDI.[33,34] Fresh stool must be processed within 6 h of donor production and can be stored at room temperature until it is mixed with approximately 250-300 mL of sterile normal sodium chloride and filtered with a filter or gauze to remove large particulate matter. The resulting filtrate is placed into 50 ml syringes for infusion into the recipient's gastrointestinal tract. Frozen fecal sample must be stored at -80 degrees Celsius in a stool bank. Frozen samples are stored at -80°C in stool banks, are thawed in a 37°C water bath, mixed with saline, and must be used within 6 hours of thawing.[30,35] The optimal volume of fecal slurry for infusion is not well-defined, but larger volumes have been associated with better outcomes in CDI patients. A systematic review on FMT in CDI patients found that infusion volumes under 50 g were linked to a four-fold higher risk of FMT failure.[36]

Patients should receive detailed counseling before the procedure. Antibiotics should be avoided for 12 to 48 hours prior to FMT, and bowel preparation should be similar to that used for other endoscopic procedure which includes a standard bowel preparation. Bowel should be cleansed of prior contaminated fecal material before the FMT to ensure a healthy graft.[37] Some studies suggest administering loperamide an hour before FMT to ensure the transplanted stool remains in the gastrointestinal tract for at least 4 to 6 hours.[38.39]

Current techniques for administering fecal material include the upper gastrointestinal route (via esophagogastroduodenoscopy, nasogastric, or nasoenteric tube), the lower GI route (via colonoscopy or retention enema), and oral capsules. Each method has its own advantages and disadvantages. Although there is no universal agreement, the colonoscopy approach to FMT is preferred over fecal enema and upper tract FMT for CDI primarily due to better patient acceptance and the limited reach of enemas, which only extend to the splenic flexure.[40] At present, there is no strong evidence supporting the best delivery route for FMT in clinical practice and the decision of the method to be used should depend on the individual clinical situation.[41] A newer technique for FMT delivery termed Colonic TET (transendoscopic enteral tubing) involves the endoscopic placement of a TET tube for fecal solution delivery. This method has been associated with 98.1% patient satisfaction and a low rate of adverse events.[42]

1. *Studies assessing role of FMT in induction of remission*

Adult patients cohort

Study by Xiang etl assessed the efficacy of faecal microbiota transplantation in treating various clinical targets associated with Crohn's disease. Patients were assessed based on seven clinical targets: abdominal pain, diarrhea, hematochezia, fever, steroid-dependence, enterocutaneous fistula, and active perianal fistula. Each clinical target was recorded as a binary outcome (yes/no) during the long-term follow-up. Steroid-dependence was assessed at 6 months,

12 months, 24 months and 36 months after FMT while other targets were assessed at 1 month, 3 months, 6 months, 12 months, 24 months and 36 months after FMT. The study enrolled 214 CD patients out of which 174 patients completed the follow-up. Significant improvements were noted in various clinical targets post-FMT. 72.7% of patients experienced relief from abdominal pain, 61.6% experienced relief from diarrhea, 76% experienced relief from hematochezia, and 70.6% experienced relief from fever at one month. Additionally, 50% of steroid-dependent patients achieved steroid-free remission. Overall, 75.3% of patients showed a clinical response at one month, with 43.7% maintaining this response and 20.1% achieving sustained remission by the end of the study. Multiple courses of FMT increased the likelihood of sustained remission, with the median frequency being 3.5 courses. No FMT-related serious adverse events occurred during the follow-up. Longer disease duration and higher Harvey–Bradshaw Index were associated with poorer responses indicating that patients in the early stage of disease or with mild disease may show a better response to FMT. This study helped to understand the effectiveness of FMT in CD focussing on targeted therapeutics beyond the traditional evaluations that use HBI or Crohn's Disease Activity Index.[43]

Study by Vaughn et al examined the effects of fecal microbial transplantation from healthy donors in patients with active Crohn's disease. This prospective, uncontrolled, open-label study included 19 participants who received a single FMT session via colonoscopy. Patients microbial diversity, mucosal T-cell phenotypes and clinical and inflammatory parameters were measured over a period of 12 weeks and safety profile assessment over 26 weeks. 58% of the patients experienced clinical response (decrease in HBI>3) while 53% of patients achieved clinical remission (HBI< 5) by week 4 after FMT. However, there were no improvements in the Crohn's Disease Endoscopic Index of Severity (CDEIS) scores or C-reactive protein (CRP) levels at 12 weeks. Microbiota analysis showed overall microbial diversity increased significantly after 8 weeks of FMT ($p=0.02$).

Responders showed a more significant increase in microbial diversity compared to non-responders (p=0.03). The microbiota of clinical responders showed a significant shift towards the microbial profile of their donors at 4 weeks (p=0.003) and 8 weeks (p=0.008) post FMT. Multiple metabolic pathways linked with gut microbiota diversity such as the serine and glutamine metabolic pathways, folic acid metabolic pathways and lipid A biosynthetic pathways were also increased in responders after FMT compared to non-responders. A significant increase was noted in the regulatory T-cells (CD4+CD25+CD127lo) in the mucosa of patients after 12 weeks of FMT (mean 5% vs. 2% at baseline, p=0.05) suggesting a potential immunomodulatory effect of FMT. However, there was no significant difference in T-cell proportions between responders and non-responders. No serious adverse events occured during the 26-week follow-up period, though mild adverse events were reported which included abdominal cramping and fever which resolved with conservative management. The study concluded that FMT was safe and effective in improving clinical outcomes but not endoscopic outcomes. Also, responders showed an increase in microbial diversity along with significant changes in metabolic pathways and mucosal T cell responses.[26]

Pediatric cohort

Study by Suskind et al investigated the effects of fecal microbial transplantation in paediatric patients with active Crohn's Disease. The study was single-centre open-label design which included 9 patients with CD with mild-to moderate disease symptoms as defined by Paediatric Crohn's Disease Activity Index (PCDAI) between 10 and 29 and aged 12 to 21. Fecal slurry was slowly administered through NG tube over a 3-minute period. At two weeks post-FMT, 77.8% (7 out of 9) of patients were in clinical remission as assessed by the Paediatric Crohn's Disease Activity Index (PCDAI). At six and twelve weeks, 55.6% (5 out of 9) of patients remained in remission without additional medical therapy. The mean PCDAI score improved significantly from a baseline of 19.7 to 6.4 at two weeks and to 8.6 at six weeks. For those

not receiving additional medical intervention, mean scores were 8.8 at six weeks and 11.1 at twelve weeks. CRP levels decreased in all except one patient, from an average of 2.4 mg/dL at baseline to 1.5 mg/dL at two weeks. Levels remained below baseline at six and twelve weeks for patients not on any additional treatment. No significant changes were noted in albumin levels or hematocrit with FMT. Stool calprotectin levels decreased or remained stable in most patients. The mean level decreased from 936 mg/L at baseline to 671 mg/L at two weeks, though levels rose for most patients by the twelve-week follow-up. Most of the adverse events were mild and self-limited, including abdominal pain (n=5), bloating (n=5), diarrhea (n=4), flatulence (n=1), and rhinorrhea(n=1). Study limitations included small sample size and open label design leading to participant bias. The study reported the efficacy of FMT in improving clinical and laboratory parameters in paediatric patients with active CD.[24]

FMT in refractory or complicated CD

Study done by He Z et al evaluated the safety and efficacy of FMT in treating Crohn's disease complicated by intraabdominal inflammatory mass. The analysis included 25 patients. Average age of enrolled patients was 36 years with male predominance (52%). Patients had a mean disease duration of 6.2 years. Most common disease location was both the ileum and colon (76%). Perianal disease was present in 72% of patients. The inflammatory masses were mainly phlegmons (88%) most commonly located in the ileum/right lower quadrant (76%), with 52% of patients also had associated fistulas. Patients who responded to the initial FMT received subsequent FMTs every three months as maintenance therapy while patients who did not show response from the initial FMT underwent a second FMT within one week. 23 patients received FMT via gastroscope into the distal duodenum under anesthesia while two patents underwent FMT using transendoscopic enteral tubing. The study reported sustained clinical remission rates of 52% at 3 months, 48% at 6 months, and 32% at 12 months, decreasing to 22.7% at 18 months. 9 patients who had

disease relapse received additional FMT. Radiological assessment done in 21 patients showed healing in 9.5% of patients, improvement in 71.4%, and stability or worsening in 19%. No severe adverse events were linked to FMT and only minor side effects such as fever, self-limiting diarrhea, and transient anal pain were reported. One patient experienced spontaneous perforation which was likely unrelated to the FMT. The study demonstrated that multiple sessions of fresh FMTs could be a viable and safe option for achieving and maintaining clinical remission in CD patients with intraabdominal inflammatory masses and the ability to avoid surgical interventions in many cases.[44]

Study done by Cui et al on fecal microbiota transplantation for refractory Crohn's disease via mid-gut. The selected cases in this study had failed or been proved not suitable to biologics, immunosuppressants, steroids, aminosalicylic acid or surgery. The study included 30 patients out of which 64.5% patients had male gender with mean age of 38 years and a mean disease duration of 7.4 years. Clinical responses were recorded over a follow-up period ranging from 6 to 15 months. Clinical improvement as assessed by HBI score was achieved in 86.7% of patients while 76.7% of patients achieved clinical remission by the first month post-FMT. Inflammation markers such as erythrocyte sedimentation rate and C-reactive protein significantly decreased. ESR showed a notable reduction one month post-FMT ($p < 0.01$) and CRP decreased within one week post-FMT ($p < 0.01$). There was an increase in serum immunoglobulin M levels one month post-FMT ($p < 0.01$) and enhanced T lymphocyte and CD4+/CD 8+ cell ratios at three days post-FMT ($p < 0.05$). However, changes in B lymphocyte, NK cells, and CD8+ cells were not significant. Plasma level of hemoglobin, albumin, total cholesterol, HDL-C and LDL-C all showed a significant increase after 3 months of FMT compared to baseline ($p < 0.05$). No serious adverse events were noted during the FMT or the follow-up period. Minor side effects included fever in 6.6% of patients and increased diarrhea in 23.3% of patients within the first six hours post-FMT. The study concluded that standardized FMT

through the mid-gut is a safe, feasible, and effective rescue therapy for patients with refractory Crohn's disease.[25]

Study by Li et al was done to investigate whether fecal microbiota transplantation can be a promising switch therapy for CD patients with prior failure of Infliximab. A total of 36 patients were included out of which 4 were excluded later on during analysis. Clinical data on Infliximab and FMT was collected for 32 patients. 15.6% of patients had primary non response, 53.1% of patients had secondary loss of response and 31.3% were intolerant to IFX. The results showed that 75.0% of patients achieved a clinical response one month after FMT while 59.4% achieved clinical remission. The clinical response rates increased over three and six months to 78.1% and 71.9% respectively, while the clinical remission rates remained steady at 62.5%. In comparison to the IFX treatment stage, the clinical response rates were 65.6% at one month, decreasing to 62.5% at three months, and 46.9% at six months. No significant difference was seen in efficacy between IFX and FMT at the three follow-up time points. Among the clinical factors related to the short term and long-term efficacy of FMT, univariate analysis revealed that the lower baseline HBI, mild disease activity, and second course of FMT before relapse significantly improved the short-term (one month) efficacy of FMT. Patients with lower baseline HBI, mild disease activity or microbiota suspension from female donors had better clinical outcomes at one year after FMT. A key finding of the study was that the incidence of adverse events was significantly lower with FMT compared to IFX($p=0.002$). 6.3% of patients experienced mild AEs after FMT such as fever and diarrhea which resolved within 24 hours without the need for medication. In contrast, 46.9% of patients undergoing IFX treatment reported AEs, with 21.9% experiencing serious AEs such as tuberculosis and drug-related immunogenicity reactions which led to treatment discontinuation in several cases. This study showed that FMT had similar short-term efficacy to IFX. Patients who received FMT had a longer duration of remission with a much lower incidence of AEs as

compared to IFX. These findings suggest that FMT could be a viable alternative treatment option for Crohn's disease patients who do not respond adequately to or cannot tolerate IFX.[45]

A case report was published by Bak et al of a 16-year-old female with refractory colonic Crohn's Disease (A1L2B1 Montreal classification for Crohn’s disease)not responding to conventional treatments such as aminosalicylates, thiopurines and biologics despite dose escalation. The patient underwent Fecal Microbiota Transplantation via and biologics gastroduodenoscopy route where the fecal slurry was infused via biopsy channel of the endoscope into the second part of duodenum over 5 minutes. The patient experienced a transient fever and abdominal pain shortly after the procedure, which resolved with conservative care. Baseline Crohn's Disease Activity Index was 394 indicating severe disease. Her CDAI score decreased by approximately 66.5 % dropping to 132 after one week. The patient was maintained on mesalamine (3.0 g/day), azathioprine (50 mg/day) and Infliximab (5 mg/ kg) every 8 weeks. The patient remained in remission in the following months. A follow-up colonoscopy after 10 months of FMT showed significant mucosal healing. Clinical remission was maintained for more than 12 months, demonstrating an approximately 75% long-term remission rate.[12]

Case report by Zhang et al demonstrated the efficacy of FMT in a 32-year-old male patient with severe ileocolonic CD refractory to conventional treatment complicated by enterocolonic fistula and a large intraperitoneal inflammatory mass. The patient underwent FMT via mid-gut by gastroscopy under anaesthesia. The patient’s CDAI score decreased approximately 57.5% (537 to 228) one week after FMT. The CDAI score further reduced by 73.4% to 143 meeting the criteria for clinical remission one month after FMT and further by 88.5% to 62 at three months post FMT. This remission was sustained over nine months. The large mass and inflammation within the fistula subsided in 4-6 weeks. The patient also gained 11 kg body weight (from 50 kg to

61 kg), and his albumin and cholesterol levels normalised. The successful reduction in size of inflammatory mass and fistula healing highlight the potential of FMT as a promising rescue therapy for severe, treatment-resistant Crohn's Disease.[46]

In the case report by Kao et al, a 26-year-old patient with Crohn's colitis who had failed immunosuppressive therapy and had steroid dependent disease received FMT. Patient underwent FMT via colonoscopy route. Patient achieved clinical remission (Harvey Bradshaw Index reduced from 9 to 0) and this remission was maintained over 4 weeks. The fecal calprotectin levels initially spiked from 3490 µg/g pre FMT to over 9000 µg/g post-FMT but then decreased to 2375 µg/g by day 12 and normalized to 189 µg/g by day 28 after the procedure. Colonic mucosa showed complete healing and colonic biopsies showed no active inflammation at second visit. Notable shift in microbial profile resembling that of the donor was seen after FMT However, the patient ultimately needed additional FMT sessions and experienced a flare six weeks after the fourth session suggesting that continous treatment may be essential for maintaining sustained remission.[47]

2. *Studies assessing FMT for maintenance of remission*

A randomized trial done by Sokol et al investigated the effectiveness of FMT in maintaining remission in patients with Crohn's disease. The study included 21 patients with active disease (Harvey Bradshaw Index > 4) who had either colonic or ileo-colonic disease location and had achieved clinical remission with oral corticosteroids. Patients were randomized to receive either FMT or a sham via colonoscopy route. The primary endpoint of donor microbiota colonization at six weeks was not met by any participants, as the donor-recipient Sorensen's index remained below 0.6 for all patients at week 6. Steroid-free clinical remission rates of 87.5% were seen in the FMT group compared to 44.4% in the sham group (p=0.23) after 10 weeks of treatment which decreased to 50.0% in FMT group versus 33.3% in sham group after 24 weeks of FMT. Notably, the FMT group exhibited a significant

reduction in CDEIS scores (p=0.03), while no significant change was found in the sham group (p=0.8) six weeks after the intervention. C-reactive protein levels remained stable in patients who received FMT (p=0.5) while it was significantly higher in the sham group (p=0.008) at six weeks post FMT. A significant increase in alpha diversity was noted in FMT group but not in sham group. Although this microbial shift was transient and the alpha diversity returned to pre FMT levels after 14 weeks. Patients who failed to engraft the donor microbiota had an early flare in disease activity similar to the sham transplantation group while patients with successful donor microbiota engraftment experienced a very low flare rates (p= 0.054). Several taxa belonging to the Gammaproteobacteria class and the Clostridiales order comprising Ruminococcus gnavus linked with disease flare were identified. Taxa that were associated with maintenance of remission included Ruminococcaceae, Coprococcus, and Desulfovibrio genus. No significant adverse events were reported during the study. Although the primary endpoint of donor microbiota colonization at 6 weeks was not achieved in the study, participants who received FMT had more chances of maintaining steroid free clinical remission and had better endoscopic outcomes. The study also showed that successful donor microbiota engraftment plays a key role in clinical success of FMT.[48]

3. *Factors influencing clinical efficacy of FMT*

A systematic review and meta-analysis by Cheng et al. evaluated the efficacy and safety of fecal microbiota transplantation (FMT) in patients with Crohn's disease. The analysis included 12 studies: one randomized controlled trial, seven cohort studies, and four case studies, involving a total of 106 CD patients who received FMT. The pooled data (I^2 = 43%) indicated that 62% of the patients achieved clinical remission while 79% of patients showed a clinical response. Notably, patients who received FMT using fresh stool had a significantly higher rates of clinical remission (0.73 CI 0.61, 0.87; I^2 = 0%) compared to the patients who received frozen stool (0.43, CI 0.22, 0.86; I^2 = 80%). Metagenomics analysis showed restoration of bacterial

flora post-FMT characterized by an increase in biodiversity, stability, and resemblance to the donor's profile in clinical responders. Sub group analysis on the route of FMT delivery and the use of antibiotics before the procedure gave no conclusive results given the limited number of studies and varying methodologies. Majority of adverse events occured were mild and self-limiting. Common side effects included bloating, abdominal pain, diarrhea, constipation, vomiting, and nausea. Fever also occurred in some cases but was typically mild and resolved on its own. No major FMT-related adverse events were reported. Two patients in one study experienced adverse events that required escalation of therapy shortly after FMT. The meta-analysis had several limitations. Most of the studies had small sample sizes and lacked control groups resulting in bias and affecting the reliability of the results. The follow-up periods in these studies were variable ranging from 4 weeks to 15 months and therefore it was difficult to assess the efficacy of FMT in long term. The studies also employed different frequencies and dosages of FMT. Standardised protocol for FMT in CD patients is an area of research and more studies with uniform methodologies are required.[49]

A metanalysis by Fang et al assessed the efficacy and safety of FMT in patients with inflammatory bowel disease including both Ulcerative colitis and Crohn's disease. 23 cohort studies were included which had a total of 319 IBD patients (225 UC and 94 CD). Analysis performed on the studies including CD patients showed that the pooled clinical remission rate was 30% in patients who received FMT with a risk of heterogeneity. The clinical remission rate differed significantly between pediatric and adult CD patients, with pediatric patients having a higher rate of 45% compared to 22% in adults. Clinical response was also significant, specific percentages for clinical response in CD patients were not detailed separately from the overall results. The analysis also indicated that patients receiving fresh stool FMT had a clinical remission rate of 36%, while those receiving frozen stool FMT had a remission rate of 28%. 63 CD patients received FMT via the upper GI

route out of which 26 patients (41%) achieved clinical remission. 20 CD patients received FMT via the lower GI route out of which 10 patients (50%) achieved clinical remission. The pooled proportion of clinical remission rates of FMT via upper GI tract was 23% and 27% for lower gastrointestinal tract, the difference being not statistically significant. The analysis reported that patients with more severe disease had a higher likelihood of achieving clinical remission after FMT compared to those with milder disease. Most adverse events reported were mild and transient, such as abdominal pain and bloating, with no serious adverse events reported. To conclude, the meta-analysis reported low pooled clinical remission rates of 30 %. Better clinical responses were seen in paediatric patients and those with more severe disease. There is paucity of current evidence on stool sample preference in FMT. The metanalysis showed that fresh stool might offer a slight advantage over frozen stool. Comparison of efficacy between the routes of FMT delivery showed that both upper and lower GI routes are equally effective for administering FMT.[3]

A study by Li et al evaluated the efficacy and timing of sequential FMT in patients with Crohn's Disease. The study included 69 patients who had undergone two FMT sessions and had shown clinical improvement after the first session. 91.3% of these patients had disease duration of more than one year. Moderate disease activity was seen in 55.1% of patients while 44.9% had severe disease. 91.3% of patients showed a clinical response while 68.1% of patients achieved clinical remission at four weeks after first FMT. The HBI scores decreased significantly 4 weeks after the first FMT in comparison to the baseline values (4.12 ± 1.69 vs. 8.51 ± 2.55 $p<0.001$). However, the efficacy of the first FMT decreased over time, with 62.3% of patients maintaining a clinical response until the second FMT which was administered at a median of 125 days after the first FMT. Despite a reduction in FMT efficacy over time, the HBI scores at the time of the second FMT remained lower than the pre-FMT scores. Follow-up data for 90.3% of patients showed that the median duration of clinical response increased to 176.5 days

after the second FMT, although the increase was not statistically significant. Microbiome analysis revealed that CD patients had significantly lower operational taxonomic unit (OTU) richness compared to healthy donors ($p = 0.0003$), although the Shannon index did not show a significant difference. Notably, responders to the first FMT demonstrated a shift in their gut microbiota toward the donor's profile, with increased alpha diversity that persisted before the second FMT, suggesting a lasting impact of FMT on the gut microbiome. The study also reported significant changes in the urinary metabolites at second FMT visit with increased levels of metabolites such as indoxyl sulphate, dimethylamine, 4- hydroxyphenylacetate and hippurate which are linked with increased gut microbiota diversity. The limitations of the study were that the patients self reported symptoms were used for the clinical response assessment rather than objective parameters like fecal calprotectin or endoscopic findings. Also, the gut microbiota analysis and urine metabolites measurement were done in limited number of patients and the effects of diet on gut microbiota and the urinary metabolome were not taken into consideration. Overall, the study demonstrated that a second FMT within four months of the first could help sustain the clinical response, indicating that sequential FMT sessions may be beneficial for long-term efficacy. It also highlighted the lasting effect of FMT on gut microbiota diversity and changes in urinary metabolites in patients with active CD.[50]

Colman et al. conducted another meta-analysis to assess the efficacy of fecal microbiota transplantation in individuals with IBD which included18 studies with a total of 122 patients. Out of 122 patients, 39 were CD patients. Pooled clinical remission (CR) rate of 60.5% (with a 95% confidence interval of 28.4% to 85.6%) was seen in CD patients receiving FMT. This CR rate was derived from four studies out of which two studies had significantly large sample size and magnitude of response to FMT. The analysis reported that FMT in CD patients had a higher efficacy compared to UC where the pooled CR rate was 22%. The analysis also identified moderate heterogeneity (Cochran's Q,

P=0.05; I2 = 37%) among the CD studies suggesting variability in the response to FMT among CD patients. The included studies varied in methodologies such as FMT delivery route, number of sessions and patient demographics which might explain the variability in clinical efficacy. There was no correlation between efficacy and number of FMT sessions performed. The metanalysis reported that younger IBD patients (aged 7 to 20 years) responded well to FMT, with a pooled CR rate of 64.1% (95% CI 10.6%-96.4%), with a moderate heterogeneity among studies (Cochran's Q, P=0.029; I2 = 44%) suggesting that age may play a role in the efficacy of FMT for IBD possibly due to differences in disease progression, immune system response, or gut microbiota composition between younger and older patients. One study reported that FMT via gastroscopy resulted in a 60% remission rate in CD patients, whereas other methods showed slightly lower effectiveness. FMT was generally well-tolerated among CD patients with no serious adverse events reported across the studies. Some patients experienced mild to moderate adverse events such as fever, abdominal discomfort, and elevated CRP levels which were self-limiting and resolved within a few days after FMT. The analysis also reported that the effectiveness of FMT varied depending on several factors, including the method of FMT delivery, the preparation of the donor stool, and the frequency of administration. The choice of donor also influenced the outcomes with some studies suggesting that using stool from a first-degree relative might result in better clinical remission rates. The meta-analysis concluded that FMT could be an effective treatment option for CD patients with a substantial portion of patients achieving clinical remission. However, the variability in methodology and results across different studies highlights the need for further research to optimize FMT protocols, including donor selection, administration methods, and frequency so as to maximise the beneficial effects of FMT.[51]

4. *Studies on effects of FMT on QOL*

Study by Wei et al investigated the impact of FMT on QoL in patients diagnosed with UC and CD. The open-label trial included fourteen IBD patients (11 UC and 3 CD patients) who underwent FMT either via colonoscopy or by way of nasojejunal tube infusion. QoL measurements were taken at baseline and four weeks post-FMT using the IBD Questionnaire (IBDQ). All of the patients noticed improvement in symptoms such as hematochezia, fecal urgency, and diarrhea after four weeks of FMT. Significant reduction in Mayo scores was seen in UC patients from 5.80 ± 1.87 to 1.50 ± 1.35 ($p < 0.01$) and a 30.8% increase in their IBD Questionnaire scores from 135.50 ± 27.18 to 177.30 ± 20.88 ($p = 0.00063$). In CD patients the mean CDAI score decreased from 345.00 ± 77.78 to 135.00 ± 7.07 ($P = 0.082$) and the mean IBDQ score increased from 107.33 ± 9.45 to 127.67 ± 14.57 ($P = 0.067$) after two weeks of FMT and to 149.00 ± 20.07 ($P = 0.024$) after four weeks of FMT. No correlation was seen between the IBDQ and Mayo score before or after FMT. The correlation between CDAI and IBDQ was not calculated. Patients attitude towards FMT was assessed which showed that 78.57% of patients were reluctant to undergo repeated FMT in a short time due to procedural discomfort. Preferences for donor stool were variable; 57.14% preferred non-relative donors. 50% of patients favoured colonoscopy over other routes of FMT administration. Few self limiting adverse events such as fever and bloating were observed. No serious adverse events were reported. The findings suggested that FMT can be a promising treatment option for improving QoL in individuals with IBD, demonstrating significant improvements in both clinical outcomes and patient-reported QoL measures.[52]

5. *Effects of FMT on microbiome*

A prospective, open-label, single-centre study by Gutin et al assessed the safety, efficacy, and microbiome profile changes associated with FMT in Crohn's disease patients. Patients with HBI of at least 3 were

enrolled while those who were pregnant, severely immunocompromised, had significant comorbidities or had concomitant Clostridium difficile infection or other enteric pathogens were excluded. 10 eligible patients received a single session of FMT via colonoscope with fecal slurry delivery starting in the terminal ileum or neo terminal ileum. Clinical improvement (decrease in Harvey-Bradshaw Index score by ≥3) was seen on 30% patients one-month after FMT while 70% did not show clinical improvement. No significant differences in baseline characteristics were found between responders and non-responders, except for disease duration (p=0.03). The SES-CD score showed no significant change in the six patients who underwent follow-up colonoscopy. Fecal bacterial 16S rRNA sequencing indicated an increase in alpha diversity in two of the three responders, while no change was observed among non-responders before or after FMT. Analysis of bacterial taxa revealed that 46 OTUs showed differences between responders and non-responders before FMT and 78 after FMT. Enterobacteriaceae and Bifidobacterium were more abundant in responders before FMT, while Lachnospiraceae and Ruminococcaceae were more predominant in non-responders. The study was terminated early due to significant adverse events in two patients which mainly included flare in disease activity. This study demonstrated modest clinical benefits of FMT in patients with CD with a potential to cause harm as seen by disease flare in 2 patients. This study highlighted that responders tended to be those with lower diversity, suggesting that FMT results in better clinical responses in CD patients with more gut dysbiosis at baseline thereby helping in patient selection for FMT.[53]

A study by Kong et investigated the effects of fecal microbiota transplantation on microbiome of Crohn's Disease patients focusing on species-level changes and their correlation with sustained remission. The findings revealed that approximately 38% of the observed microbial shifts were attributed to the replacement of strains from the patient's original microbiota with those from the donor as determined

through metagenomic analysis. The study also highlighted significant variation in the success of donor species engraftment across different patients. Three patients had more than 40% species transmission from their donors, with one patient showing the engraftment of 8 out of 19 species. Among the species that successfully engrafted were *Alistipes putredinis* and butyrate producing *Coprococcus comes*, In contrast, some species showed resistance to engraftment. *Ruminococci* species which are commonly associated with a healthy gut were prevalent in microbiome of CD patients but did not engraft in any of the patients. Similarly, *Faecalibacterium prausnitzii*, which is considered a beneficial bacteria due to its anti-inflammatory properties did not consistently engraft despite its presence in both donors and patients. The study also reported long-term coexistence of donor and patient strains. This coexistence suggested that even when donor strains do not completely replace patient strains, they can still establish themselves and potentially contribute to the overall microbiome composition and function. Changes in the microbial community's functional potential after FMT were also observed which included an increase in a creatinine degradation pathway. A decrease in community diversity was observed at the first time point after relapse characterised by loss/reduction of commensal microbial community members during CD disease activity and decrease in community potential for anaerobic energy metabolism. The research demonstrated that FMT significantly changed the patients' microbiomes even for those who were classified as FMT failures where there was no large-scale shift towards the donor's microbiota. These patients still had microbial changes at the strain level, indicating that broader profiling methods are crucial for fully understanding the outcomes of FMT. This study also reported whether engraftment in FMT is associated with maintenance of remission. Engraftment of Proteobacteria ($p = 0.028$) and Bacteroidetes ($p = 0.032$) were associated with the likelihood of relapse. The study found out that Roseburia intestinalis has the strongest association with probability of relapse although this species was transmitted poorly in FMT with only one engraftment event. Also it was not significantly

associated with FMT at the strain level (p = 1). The variation in strain engraftment shows that personalized approaches in selecting FMT donors could be more effective in maintenance of remission in CD patients. The study also reported that the post-FMT perturbation to the patient's microbiome settles into its long-term state starting from the 14-week time point. Future research is required aiming to optimize FMT as a therapeutic strategy by tailoring donor selection to individual patient needs and microbiome characteristics.[54]

6. *Comparison of FMT delivery routes*

Study by Yang et al investigated the efficacy and safety profile of FMT in Crohn's disease patients comparing FMT delivery via gastroscopy and colonoscopy routes. The study included 27 patients with active Crohn's disease who were randomly assigned to receive FMT through either the upper or lower gastrointestinal route. Second session of FMT was performed one week later. Clinical assessment and serum testing were done at multiple intervals (1st, 2nd, 4th, 6th, and 8th week post-FMT). Endoscopic assessment was done at baseline and after 8 weeks of FMT. 66.7% of the patients achieved clinical remission while 77.8% of the patients showed clinical response by the end of the study. The clinical remission rates between the gastroscopy (69.2%) and colonoscopy (64.3%) groups were not significantly different. The study also compared outcomes between patients who received FMT from related versus unrelated donors. Those receiving FMT from related donors had a higher remission rate (73.7%) compared to those with unrelated donors (50%), though this difference was also not statistically significant. Microbiota diversity was assessed using operational taxonomic units count and the Shannon diversity index. Prior to FMT, patients had significantly lower microbiota diversity compared to donors. However, by the second week post-FMT, the Shannon diversity index (increasing from a baseline of 2.05 to 3.18) and OTU count (from 117 to 210) had significantly improved indicating that FMT successfully altered the gut microbiota to more closely resemble that of the donors. Mild adverse events were reported in 76.9% of patients in

the gastroscopy group and 64.3% in the colonoscopy group with no statistically significant difference between the two groups. The most common adverse events were diarrhea in 14.3% of patients in the gastroscopy group and 57.1% in the colonoscopy group, abdominal pain was reported by 21.4% of patients in the gastroscopy group and 14.3% in the colonoscopy group. Most adverse events were self-limited and resolved spontaneously within 24 hours. None of the patients had serious infections or complications during or after the procedure. To summarize, this study showed that FMT is an effective and safe treatment for inducing clinical remission in Crohn's disease patients, with no significant differences in efficacy or safety between the upper and lower GI delivery methods. Significant changes in OTU counts and Shannon diversity index were seen denoting successful modulation of patient's gut microbiota after FMT. The study also reported the potential benefit of using related donors for FMT which showed better response rates in this study. Further research is required to optimize the procedure and evaluate long-term outcomes.[55]

7. *Safety profile of FMT*

Study by Wang et al evaluated the long-term safety profile of fecal microbiota transplantation for Crohn's Disease. A total of 139 patients were included who underwent a total of 184 FMT procedures. All patients underwent FMT delivery through the mid-gut. 13.6% of the procedures resulted in mild adverse events within the first week post FMT. A 1-month cut-off was suggested to define short-term and long-term AEs of FMT. The most common AEs included increased frequency of defecation (13 cases), fever (8 cases), abdominal pain (5 cases), flatulence (2 cases), and individual reports of hematochezia, vomiting, bloating, and herpes zoster. 84% of AEs were classified as Grade 1 that is they were self-limited. 16% of patients required medication to manage their symptoms. The study found that the method used to prepare the fecal microbiota significantly influenced the incidence of AEs. Manual preparation methods were associated with a higher AE rate of 21.7% compared to 8.7% for automatic preparation

methods. The study found no significant differences in FMT efficacy when comparing different preparation methods. However, patients who experienced adverse events had lower clinical response (45%) and remission rates (20%) compared to those without AEs, who had response and remission rates of 75.6% and 63%, respectively. There were several limitations in the study including nonrandomized design, no microbiota sequencing, only mid-gut delivery of FMT and disease activity assessment by HBI score which has a limited correlation to the CD endoscopic activity index and SES-CD score. The study also reported that the difference in methodologies of fecal slurry preparation can have impact on adverse event occurrence but that it did not affect the clinical efficacy of the procedure.[56]

MATERIALS AND METHODS

Study design

This was a randomised, double-blind, placebo-controlled trial of FMT in patients with mild to moderate active CD conducted at Dayanand Medical College and Hospital, Ludhiana, India.

Disease definition, classification and assessment of disease activity

Adult patients with established diagnosis of CD (based on the European Crohn's and Colitis Organisation [ECCO] and the European Society of Gastrointestinal and Abdominal Radiology [ESGAR] guidelines) were included. Montreal classification was used for classifying CD. Clinical disease activity was assessed by Crohn's disease activity index.

Clinical remission was defined as CDAI score < 150 points, while mild to moderate disease was defined as CDAI 150-449 points. Clinical response was defined as a decline in CDAI by >100 points. Endoscopic disease activity was assessed by simple endoscopic score for CD (SES-CD). SES CD ≥6 indicated active disease. Endoscopic response was defined by a 50% reduction in the SES-CD score from baseline.

Table 1: Montreal Classification for adult CD

Parameters		**Description**
Age at diagnosis	A1	Age less than 16 years
	A2	Age between 17 and 40 years
	A3	Age above 40 years
Location	L1	Ileal
	L2	Colonic
	L3	Ileocolonic
	L4	Isolated upper GI disease*
Behavior	B1	Non-stricturing, non-penetrating
	B2	Stricturing
	B3	Penetrating
	P	Perianal disease†

*L4 is a modifier that can be added to L1-3 when concomitant upper gastrointestinal disease is present. † "P" is added to B1-B3 when concomitant perianal disease is present.

Table 2 : Crohn's Disease Activity Index (CDAI)	
Clinical or laboratory variable	Weighting factor
Number of liquid stools or soft stools each day for last 7 days	2
Abdominal pain (graded from 0 to 3 based on severity) each day for last 7 days	5
General well assessed subjectively from 0 (well) to 4 (terrible) each day for 7 days	7
Complications*	20
Use of diphenoxylate or opiates for diarrhea	30
An abdominal mass (0 for none, 2 for questionable, 5 for definite)	10
Absolute deviation of haematocrit from 47% in men and 42% in women	6
Percentage deviation from standard weight	1
*One point is given for each set of complications: arthralgia or frank arthritis, inflammation of the iris, sclera or uveitis, erythema nodosum, pyoderma gangrenosum, aphthous ulcers, anal fissures, fistulas, abscesses, other fistulas, and fever (>100 F) during the previous week	

Table 3: Simple endoscopic score for Crohn's disease				
Variable	Score			
	0	1	2	3
Size of ulcers	None	Aphthous ulcers (diameter 0.1-0.5 cm)	Large ulcers (diameter 0.5-2 cm)	Very large ulcers (diameter > 2 cm)
Ulcerated surface	None	<10%	10-30%	>30%
Affected surface	Unaffected segment	<50%	50-75%	>75%
Presence of narrowings	None	Single, can be passed	Multiple, can be passed	Cannot be passed
Total SES CD : sum pf the values of the four variables for the 5 bowel segments (ileum, right colon, transverse colon, left colon and rectum) SES CD ≥6 indicates active disease. Endoscopic response is defined by a 50% reduction in the SES-CD score from baseline.				

Inclusion criteria

1. Patients with mild to moderate active CD, defined as CDAI 150-449 and SES CD ≥6 (endoscopic assessment performed within 12 weeks of screening)
2. Ileal/ colonic / Ileocolonic disease location.
3. Inflammatory or stricturing disease behaviour.
4. Diagnosis (endoscopic or radiographic and histological) of CD at least 6 months prior to enrolment into the study
5. Age ≥ 18 years
6. Subjects who were willing and able to comply with treatment plan, laboratory tests.
7. Subjects who were willing to provide a written informed consent.

Exclusion criteria

1. Patients with severe CD (CDAI>450)
2. Fistulizing disease behavior
3. Perianal fistulizing disease
4. SES CD <6
5. Subjects without previous treatment for IBD (i.e. treatment-naïve)
6. Active or latent or inadequately treated infections (including Clostridiodes difficile, Cytomegalovirus, Mycobacterium tuberculosis).
7. Subjects displaying clinical signs of fulminant colitis or toxic megacolon
8. Subjects who received antibiotics or probiotics within 4 weeks of screening
9. Subjects who have been vaccinated with live or attenuated vaccine within 6 weeks of baseline or scheduled to receive these vaccines during study period or within 6 weeks after last FMT session
10. Subjects with malignancies or a history of malignancies
11. Subjects with current or recent history of severe, progressive, or uncontrolled renal, hepatic, haematological, gastrointestinal, metabolic, endocrine, pulmonary, cardiac, neurological disease.
12. Pregnant females
13. Age <18 years

Permitted therapies

1. Oral mesalazine (stable dose for ≥4 weeks)
2. Thiopurines or methotrexate (stable dose for ≥4 weeks)
3. Oral prednisolone or equivalent (dose ≤20 mg daily).
4. Patients were required to continue stable doses of all CD therapies during the trial period except for prednisolone, which had a mandated tapering of 5 mg weekly to ensure corticosteroid-free status by week 4.

Selection and Screening of Stool Donor

A preliminary interview of the prospective donor was performed by a structured questionnaire that will investigate several risk factors to minimize the risk of transferring infections or adverse gut microbiota profile. In the medical interview screening, potential donors werw inquired about the use of drugs that can alter gut microbiota, known history or risk behaviors for infectious disease, and for disorders potentially associated with the disruption of gut microbiota.

Donor stool was evaluated for various enteric pathogens, which included parasites like *Cryptosporidium*, *Entamoeba histolytica*, *Giardia*, and *Cyclospora*. Screening for bacteria such as *Salmonella*, *Vibrio*, *Plesiomonas*, *Shigella*, *Escherichia coli*, *Campylobacter*, *Yersinia*, and *Clostridioides difficile*, along with viruses like adenovirus, astrovirus, norovirus, rotavirus, and sapovirus was also done. Stool cultures were specifically performed for *Salmonella*, *Shigella*, *Escherichia coli*, *Campylobacter*, and *Yersinia*. Additionally, serological testing was conducted on donors for hepatitis A, B, C, E, human immunodeficiency virus, cytomegalovirus and syphilis. A brief questionnaire was administered on the day of donation to assess for any recent harmful events.

Minimum criteria for donor selection

- Age > 20 years and < 50 years

- BMI between 18-25 kg/m^2 (body mass index is defined as the body mass divided by the square of the body height)
- No co-morbid illness associated with gut microbial dysbiosis
- Regular bowel movements

Randomization

Patients were randomized in a 1:1 ratio to receive either FMT or placebo, using a pre-established computer-generated randomisation list. The individual who performed the randomisation was not involved in the trial.

Sample size

We expected a true mean difference of 30% in the treatment and the control group. Using α error rate of 5% and 80% power of the study, δ (clinically meaningful difference) of 20 (response rates to FMT vary between 50-80%) and a 30% dropout rate; an estimated 36 patients (18 in each group) were required for inclusion.

Preparation and administration of fecal slurry

A freshly collected stool sample was visually examined, and only formed stools (Bristol stool scale categories 3 and 4) that showed no visible blood or mucus were accepted. The sample was then diluted with preservative-free normal saline and homogenized using a blender (Stomacher® 400 circulator; Seward Ltd, Worthing, UK) until it achieved a liquid consistency. Universal precautions were followed and separate disposable bags were used for each patient. The stool slurry was filtered to remove particulate matter and filled into four 50 mL syringes. It was then infused into the terminal ileum and cecum via colonoscopy within six hours of stool collection and one hour after slurry preparation. Recipients were advised to retain the stool slurry for as long as possible and were monitored for six hours to assess stool retention time and any immediate post-procedure adverse events. FMT sessions were done at 0,4 ,12 and 20 weeks.

Investigations

Blood samples were collected at baseline for complete blood counts (including hemoglobin, leucocyte count and platelets), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and renal and liver function tests. Fecal calprotectin was performed at baseline and at week 24.

STATISTICAL ANALYSIS

The statistical analysis followed the intention-to-treat approach and results were reported according to the CONSORT statement recommendations. Patients' characteristics were described for the total cohort and for each group of randomization. Qualitative data was expressed as number (%) and quantitative data was expressed as medians and interquartile range (IQR). The primary and secondary outcomes were compared between the two groups with a Fisher's exact test. Quantitative variables were compared using a non-parametric Wilcoxon rank-sum tests.

OBSERVATION AND RESULTS

Table 4: Baseline characteristics

Parameters	Intervention		p value
	FMT (n = 11)	Placebo (n = 9)	
Age (Years)	35.82 ± 11.89	44.11 ± 7.75	0.160
Age			0.297
18-30 Years	4 (36.4%)	0 (0.0%)	
31-40 Years	3 (27.3%)	4 (44.4%)	
41-50 Years	3 (27.3%)	3 (33.3%)	
51-60 Years	1 (9.1%)	2 (22.2%)	
Gender			1.000
Male	5 (45.5%)	5 (55.6%)	
Female	6 (54.5%)	4 (44.4%)	
Age at Diagnosis (Years)	30.55 ± 8.45	40.89 ± 9.89	0.044
Disease Duration (Years)	3.68 ± 5.49	3.01 ± 4.31	0.356

The study included a total of 20 participants. 11 (55.0%) participants received FMT while 9 (45.0%) participants received placebo. Out of 20 patients, 16 patients completed the follow up, 3 patients withdrew consent after 2 sessions of FMT and 1 patient dropped out after first session of FMT due to disease flare.

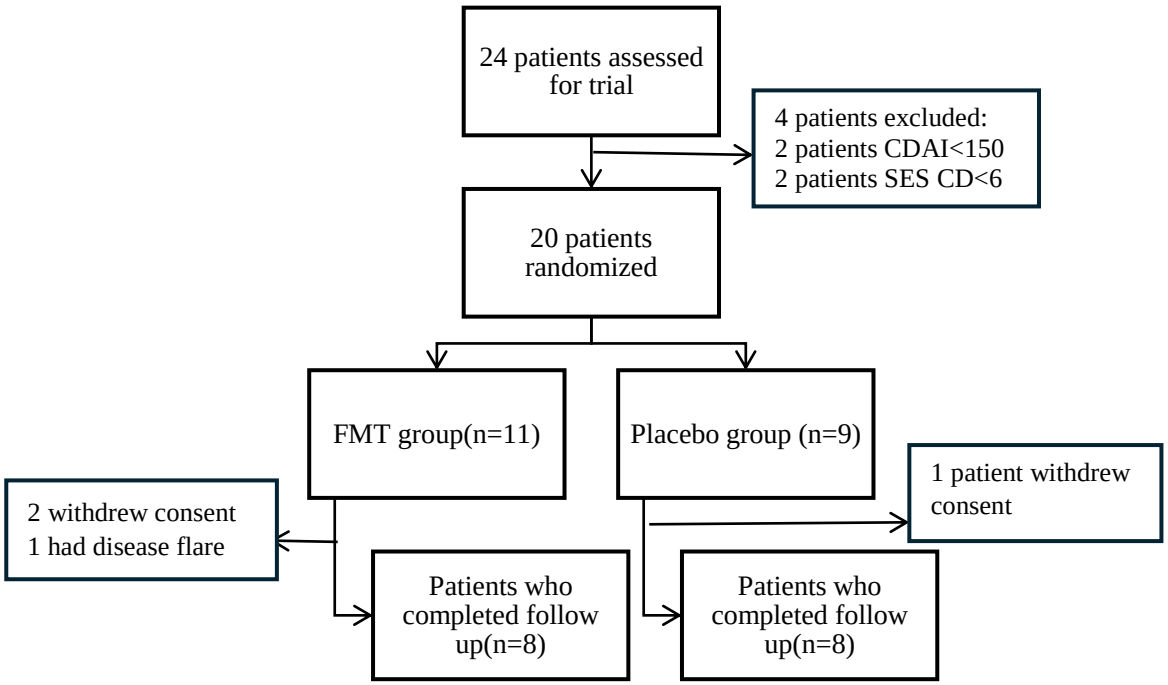


Figure 1: Flow diagram of the study

Age of participants in two groups

The mean age of participants was 39.55±10.85 years. The mean age in the FMT group was 35.82 ± 11.89 years (range 18-56). The mean age in the placebo group was 44.11± 7.75 years (range 35-57). There was no significant difference between the groups in terms of age (p = 0.160). In the FMT group, 36.4% of the participants were in age group 18-30 years, 27.3% of the participants were in 31-40 years age group, 27.3% of the participants were in 41-50 years age group and 9.1% of the participants were in 51-60 years age group. In placebo group, none of the participants were in 18-30 years age group, 44.4% of the participants were in 31-40 years age group, 33.3% of the participants were in 41-50 years age group and 22.2% of the participants were in 51-60 years age group. There was no significant difference between the various groups in terms of distribution of age ($\chi 2 = 4.319$, p = 0.297).

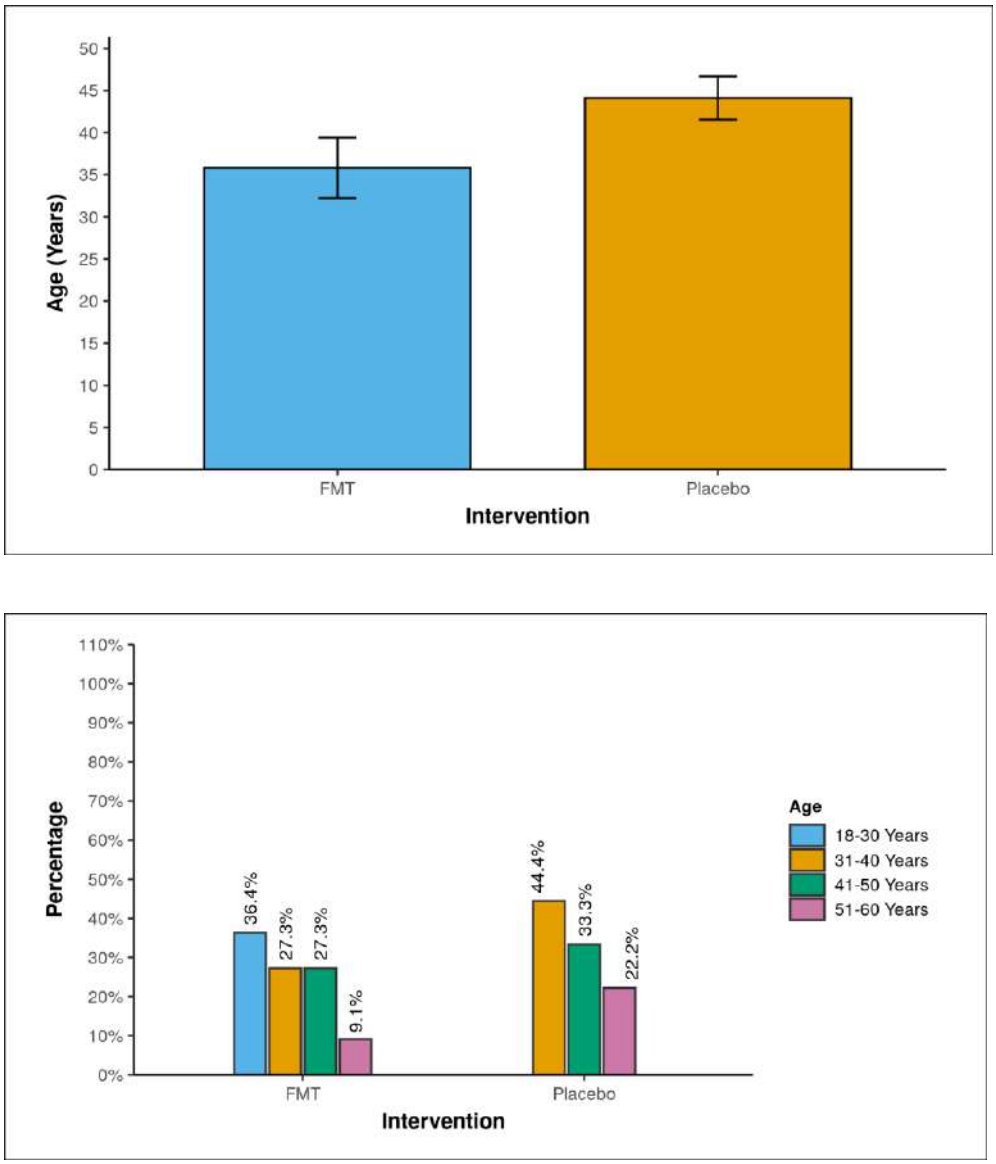


Figure 2: Age distribution in two groups

Distribution of the participants in terms of age at diagnosis

The age at diagnosis ranged between 15-40 years in the FMT group (median: 33 years, IQR = 24.5-37.5) while that in placebo group ranged between 26-56 years (median: 40 years, IQR = 34-46). A significant difference was noted between the median age at diagnosis amongst the two groups. (p = 0.044)

Table 5: Age at diagnosis in two groups

Age at Diagnosis (Years)	Intervention		Wilcoxon-Mann-Whitney U Test	
	FMT	Placebo	W	p value
Mean (SD)	30.55 (8.45)	40.89 (9.89)	22.500	0.044
Median (IQR)	33 (24.5-37.5)	40 (34-46)		
Min - Max	15 - 40	26 - 56		

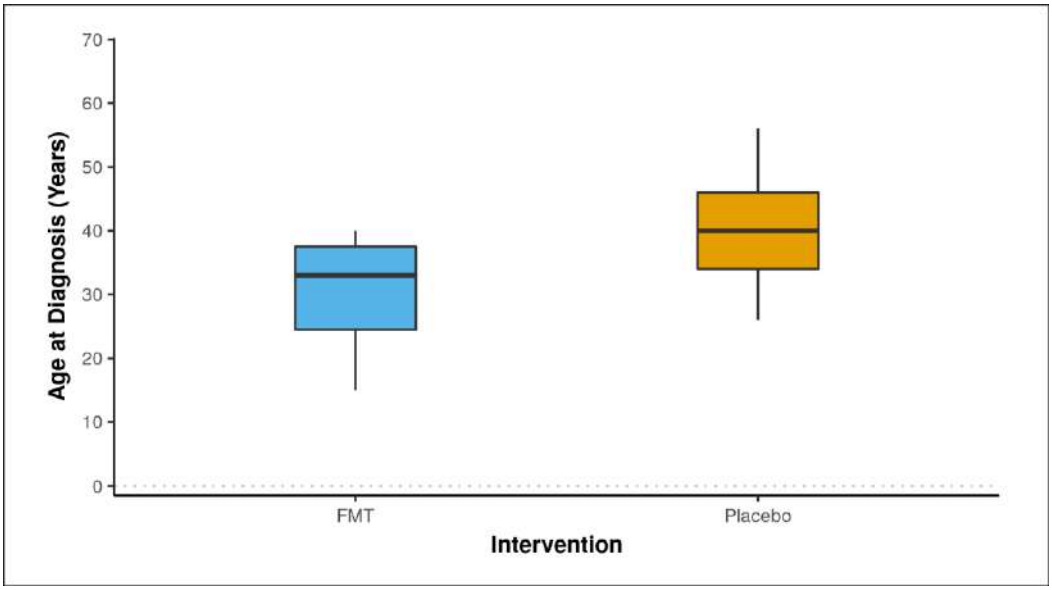


Figure 3: Distribution of age at diagnosis in two groups

Distribution of disease duration in two groups

The duration of disease ranged from 1-20 years in the FMT group (median:2.5 years, IQR = 1-3) while in placebo group it ranged between 0.33-14 years (median: 2 years, IQR= 0.6-3). There was no significant difference between the two groups in terms of disease duration (p = 0.356).

Table 6: Disease duration in two groups

Disease Duration (Years)	Intervention		Wilcoxon-Mann-Whitney U Test	
	FMT	Placebo	W	p value
Mean (SD)	3.68 (5.49)	3.01 (4.31)	62.000	0.356
Median (IQR)	2.5 (1-3)	2 (0.6-3)		
Min - Max	1 - 20	0.33 - 14		

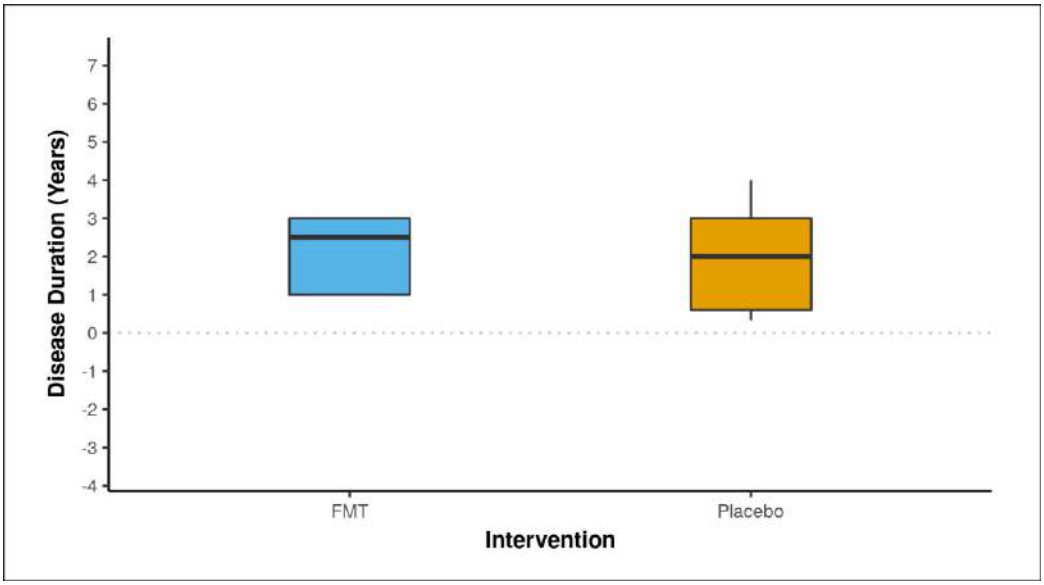


Figure 4: Distribution of disease duration in two groups

Baseline parameters

Table 7: Baseline characteristics-2

Parameters	Intervention		p value
	FMT (n = 11)	Placebo (n = 9)	
BMI (kg/m²)	22.93 ± 3.74	20.79 ± 3.90	0.270
Comorbidities			
Diabetes Mellitus	1 (9.1%)	1 (11.1%)	1.000
Hypertension	0 (0.0%)	1 (11.1%)	0.450
CAD	0 (0.0%)	0 (0.0%)	1.000
COAD	0 (0.0%)	0 (0.0%)	1.000
CVA	0 (0.0%)	0 (0.0%)	1.000
Hypothyroidism	1 (9.1%)	0 (0.0%)	1.000
Hyperthyroidism	0 (0.0%)	0 (0.0%)	1.000
Addictions			
Smoking	0 (0.0%)	0 (0.0%)	1.000
Alcohol	2 (18.2%)	1 (11.1%)	1.000
Opium	1 (9.1%)	0 (0.0%)	1.000
IVDU	0 (0.0%)	0 (0.0%)	1.000
Appendicectomy	0 (0.0%)	0 (0.0%)	1.000
Disease Location			0.842
L 1	3 (27.3%)	4 (44.4%)	
L 2	6 (54.5%)	3 (33.3%)	
L 3	2 (18.2%)	2 (22.2%)	
Disease Behaviour			0.074
B 1	11 (100.0%)	6 (66.7%)	
B 2	0 (0.0%)	3 (33.3%)	
CDAI	172.27 ± 36.66	182.56 ± 33.67	0.517
HBI	3.18 ± 2.23	3.89 ± 1.69	0.227
SES CD	9.73 ± 5.98	8.56 ± 4.69	0.647
Slurry Retention Time (Hours)	3.82 ± 1.17	4.00 ± 0.66	0.694
Previous treatment			
IV Steroids	3 (27.3%)	0 (0.0%)	0.218
Oral Prednisolone	5 (45.5%)	4 (44.4%)	1.000
Oral Budesonide	5 (45.5%)	1 (11.1%)	0.157
5-ASA	3.25 ± 0.37	2.51 ± 1.07	0.047
AZA/ 6- MP	20.45 ± 36.77	25.00 ± 30.62	0.654
TOFA	4.55 ± 8.20	0.00 ± 0.00	0.112
IFX	0 (0.0%)	0 (0.0%)	1.000

Parameters	Intervention		p value
	FMT (n = 11)	Placebo (n = 9)	
ADA	0 (0.0%)	1 (11.1%)	0.450
VDZ	0 (0.0%)	0 (0.0%)	1.000
MTX	0 (0.0%)	0 (0.0%)	1.000
Investigations			
Haemoglobin (g/dL)	10.32 ± 1.82	9.56 ± 2.14	0.196
PCV (%)	32.17 ± 6.03	30.08 ± 5.19	0.223
TLC (/mm³)	7463.64 ± 2010.11	6933.33 ± 1882.82	0.543
Platelet Count (10^3/mm³)	298.91 ± 130.56	241.44 ± 95.80	0.447
AST (U/L)	43.00 ± 49.36	26.44 ± 11.18	0.790
ALT (U/L)	61.18 ± 113.91	28.33 ± 16.63	0.879
ALP (U/L)	96.91 ± 19.21	87.44 ± 20.79	0.342
CRP (mg/dL)	15.78 ± 31.29	8.74 ± 10.91	0.766
S. Creatinine (mg/dL)	0.82 ± 0.15	0.83 ± 0.24	0.970
Fecal Calprotectin (µg/g)	976.09 ± 1117.81	426.44 ± 189.67	0.790

Baseline Body Mass Index (BMI) in two groups

The BMI in the FMT group ranged between 17.4 - 30.1 kg/m^2 with a mean of 22.93 ± 3.74 kg/m^2 and that in the placebo group ranged between 13.7 - 26.3 kg/m² with a mean of 20.79 ± 3.90 kg/m². The difference between the two groups was not statistically significant (p = 0.270).

Table 8: Baseline BMI in two groups

BMI (kg/m²)	Intervention		Wilcoxon-Mann-Whitney U Test	
	FMT	Placebo	W	p value
Mean (SD)	22.93 (3.74)	20.79 (3.90)	64.500	0.270
Median (IQR)	21.8 (20.4-25.5)	20.4 (19-23.5)		
Min - Max	17.4 - 30.1	13.7 - 26.3		

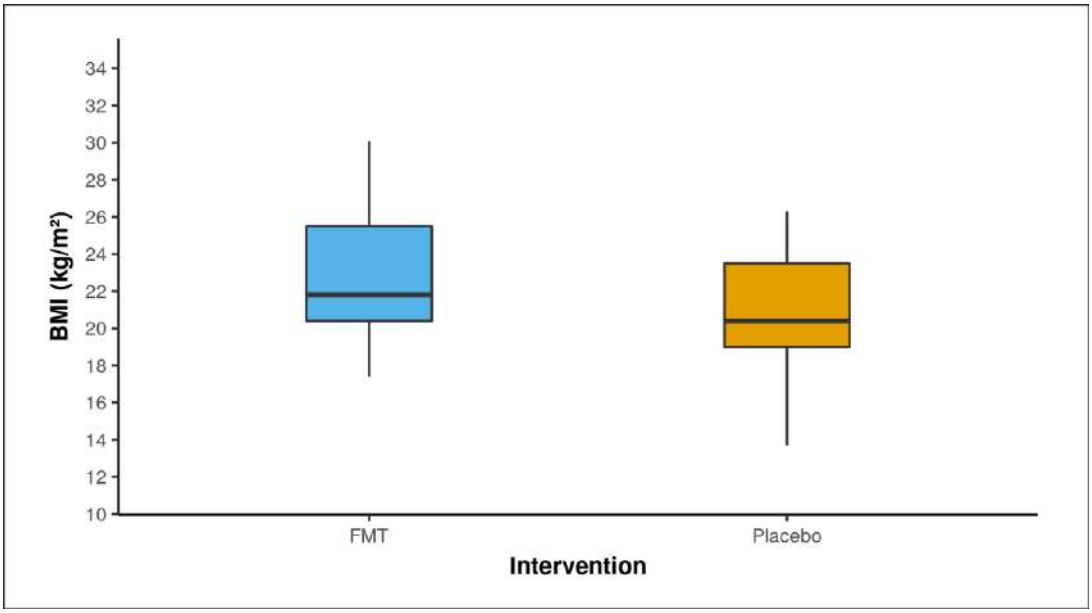


Figure 5: Baseline BMI distribution

History of comorbidities in two groups

Among comorbidities, 9.1% of the participants in the FMT group had history of diabetes mellitus whereas 11.1% of the participants in placebo group had history of diabetes mellitus. (p=1.000). 11.1% of the participants in the placebo group had history of hypertension while none of the patients had positive history in FMT group (p=0.450). 9.1% of the participants in the FMT group had history of hypothyroidism while none of the patients had positive history in placebo group(p=1.000). None of the patients had history of hyperthyroidism, cerebrovascular accident, chronic obstructive airway disease and coronary artery disease.

History of addictions in two groups

Among addictions, Alcohol consumption was seen in 18.2 % (n=2) in FMT group and 11.1 %(n=1) in the placebo group(p=1.000) Opium consumption was seen in 9.1 % (n=1) in FMT group while it was not seen in placebo group(p=1.000). None of the patients had history of smoking or IV drug use.

None of the patients had history of appendicectomy.

Distribution of disease location in two groups

Regarding disease location, in the FMT group 27.3% of the participants had ileal disease, 54.5% of the participants had colonic disease and 18.2% of the participants had ileocolonic disease. In the placebo group, 44.4% of the participants had ileal disease, 33.3% of the participants had colonic disease and 22.2% of the participants had ileocolonic disease. There was no significant difference between the two groups in terms of distribution of disease location ($\chi 2 = 0.952$, $p = 0.842$).

Table 9: Distribution of disease location

Disease Location	Intervention			Fisher's Exact Test	
	FMT	Placebo	Total	χ2	P Value
Ileal (L 1)	3 (27.3%)	4 (44.4%)	7 (35.0%)	0.952	0.842
Colonic (L 2)	6 (54.5%)	3 (33.3%)	9 (45.0%)		
Ileocolonic (L 3)	2 (18.2%)	2 (22.2%)	4 (20.0%)		
Total	11 (100.0%)	9 (100.0%)	20 (100.0%)		

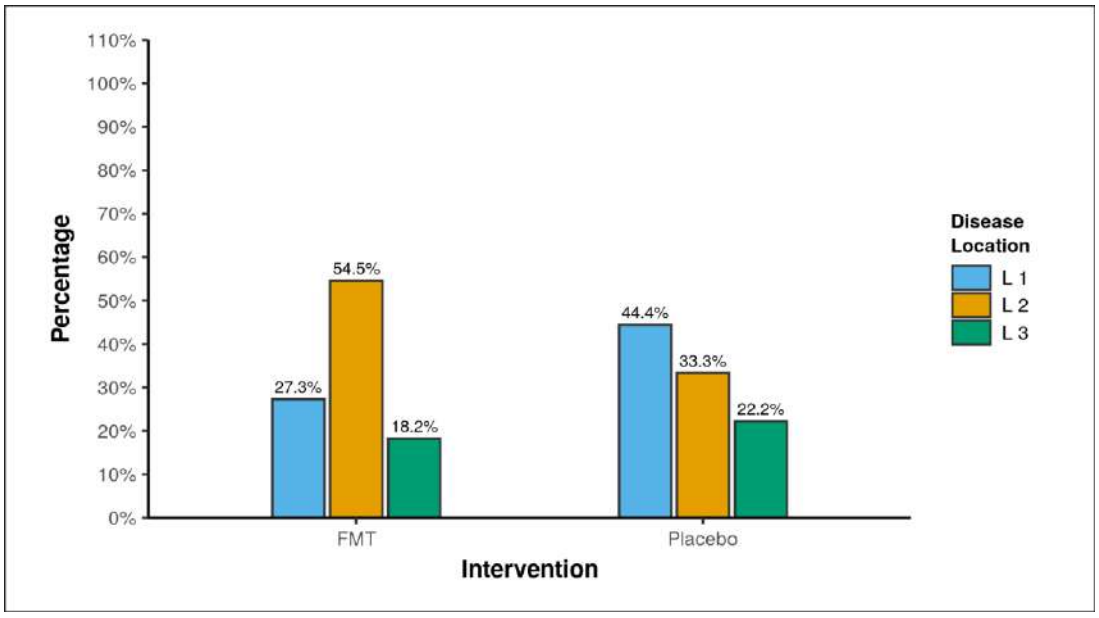


Figure 6: Distribution of Disease Location

Distribution of disease behaviour in two groups

All of the participants in the FMT group had non stricturing non penetrating disease phenotype(B1). 66.7% of the participants in the placebo group had B 1 disease behaviour and 33.3% of the participants in the placebo group had stricturing disease behaviour(B2). There was no significant difference between the two groups in terms of distribution of disease behaviour ($\chi 2 = 4.314$, $p = 0.074$).

Table 10: Distribution of disease behaviour in two groups

Disease Behaviour	Intervention			Fisher's Exact Test	
	FMT	Placebo	Total	χ2	P Value
B 1	11 (100.0%)	6 (66.7%)	17 (85.0%)	4.314	0.074
B 2	0 (0.0%)	3 (33.3%)	3 (15.0%)		
Total	11 (100.0%)	9 (100.0%)	20 (100.0%)		

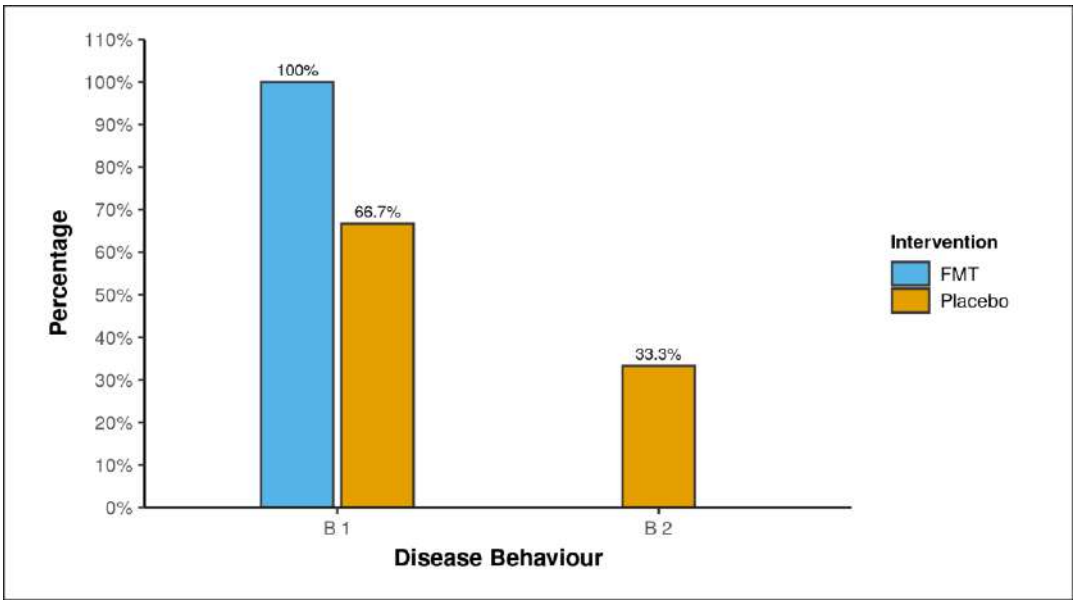


Figure 7: Disease behaviour distribution

Comparison of baseline CDAI in two groups

The mean CDAI at 1st visit in the FMT group was 172.27±36.66. The mean CDAI at 1st visit in the placebo group was 182.56±33.67. The CDAI at 1st visit in the FMT group ranged from 151 - 272. The CDAI at 1st visit in the placebo group ranged from 151 - 240. There was no significant difference between the groups in terms of baseline CDAI (p = 0.517).

Table 11: Association between Intervention and Baseline CDAI

CDAI (Baseline)	Intervention		Wilcoxon-Mann-Whitney U Test	
	FMT	Placebo	W	p value
Mean (SD)	172.27 (36.66)	182.56 (33.67)	40.500	0.517
Median (IQR)	157 (152.5-170.5)	182 (153-195)		
Min - Max	151 - 272	151 - 240		

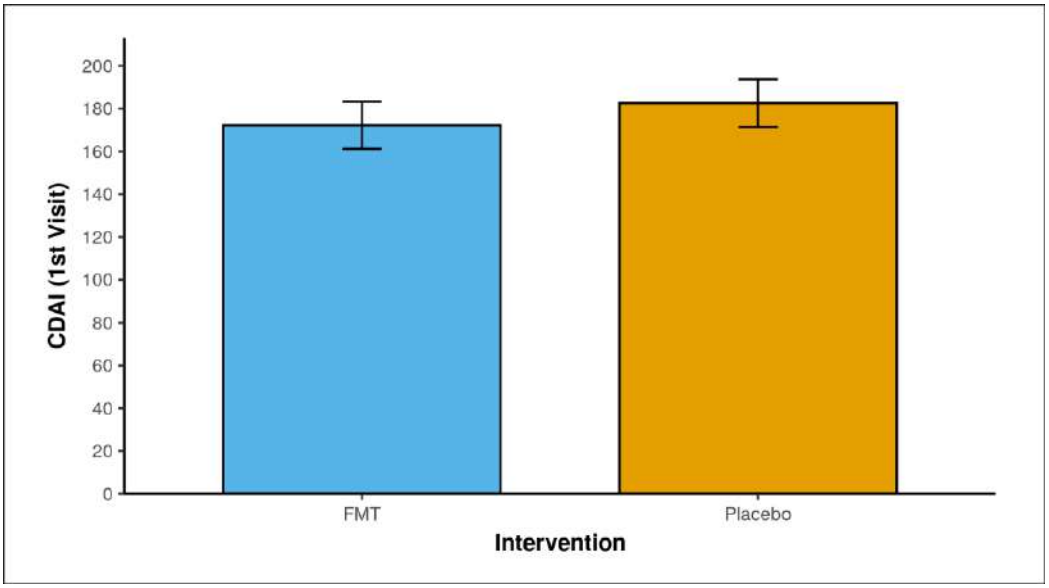


Figure 8: Baseline CDAI in two groups

Comparison of baseline HBI in two groups

The mean HBI at 1st visit in the FMT group was 3.18±2.23. The mean HBI at 1st visit in the placebo group was 3.89±1.69. The HBI at 1st visit in the FMT group ranged from 1 - 8. The HBI at 1st visit in the placebo group ranged from 2 - 6. There was no significant difference between the groups in terms of baseline HBI (p = 0.227).

Table 12: Association between Intervention and Baseline HBI

HBI (Baseline)	Intervention		Wilcoxon-Mann-Whitney U Test	
	FMT	Placebo	W	p value
Mean (SD)	3.18 (2.23)	3.89 (1.69)	33.500	0.227
Median (IQR)	2 (2-4)	3 (3-6)		
Min - Max	1 - 8	2 - 6		

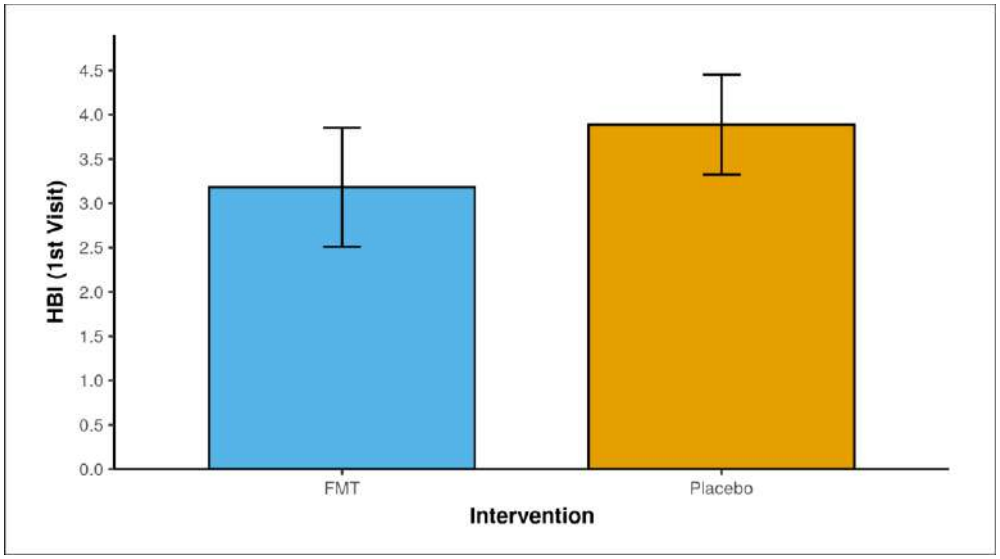


Figure 9: Baseline HBI in two groups

Comparison of baseline SES-CD in both groups

The mean SES CD at 1st visit in FMT group was 9.73±5.98. The mean SES CD at 1st visit in the placebo group was 8.56±4.69. The SES CD at 1st visit in the FMT group ranged from 0 - 20. The SES CD at 1st visit in the placebo group ranged from 3 - 18. There was no significant difference between the groups in terms of baseline SES CD (p = 0.647).

Table 13: **Association between Intervention and Baseline HBI**

SES CD (Baseline)	Intervention		Wilcoxon-Mann-Whitney U Test	
	FMT	Placebo	W	p value
Mean (SD)	9.73 (5.98)	8.56 (4.69)	56.000	0.647
Median (IQR)	10 (6-13.5)	8 (6-10)		
Min - Max	0 - 20	3 - 18		

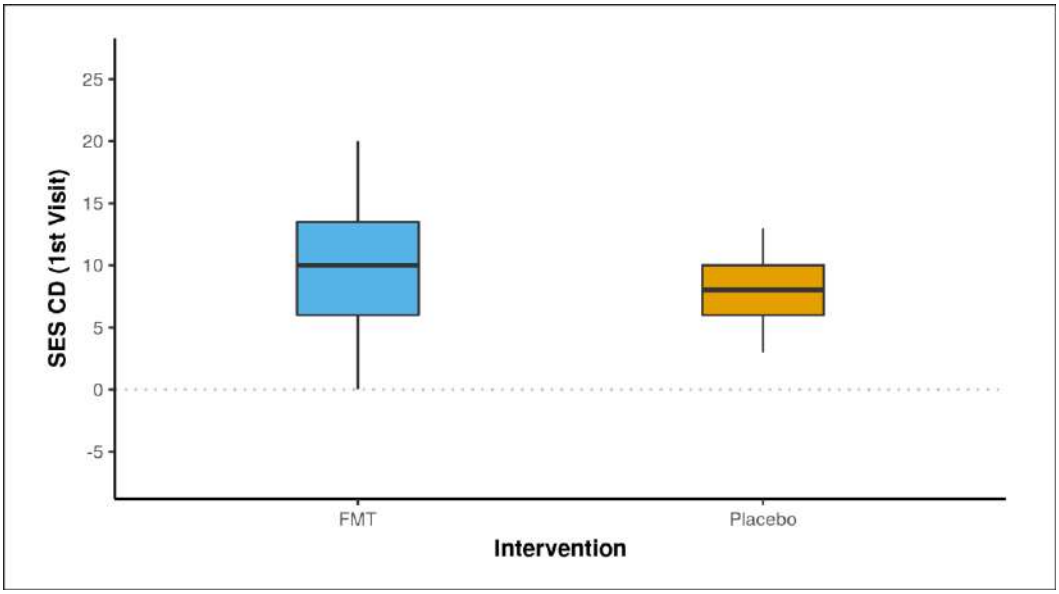


Figure 10: Baseline SES CD in two groups

Previous treatment use in two groups

Intravenous steroids had been used previously in 27.3% of patients in the FMT group while none of the participants in the placebo group had been given intravenous steroids in the past. There was no significant difference between the various groups in terms of history of previous treatment with IV steroids ($\chi 2 = 2.888$, $p = 0.218$).

Table 14: Previous Treatment with IV steroids

Previous Treatment: IV Steroids	Intervention			Fisher's Exact Test	
	FMT	Placebo	Total	χ2	P Value
Yes	3 (27.3%)	0 (0.0%)	3 (15.0%)	2.888	0.218
No	8 (72.7%)	9 (100.0%)	17 (85.0%)		
Total	11 (100.0%)	9 (100.0%)	20 (100.0%)		

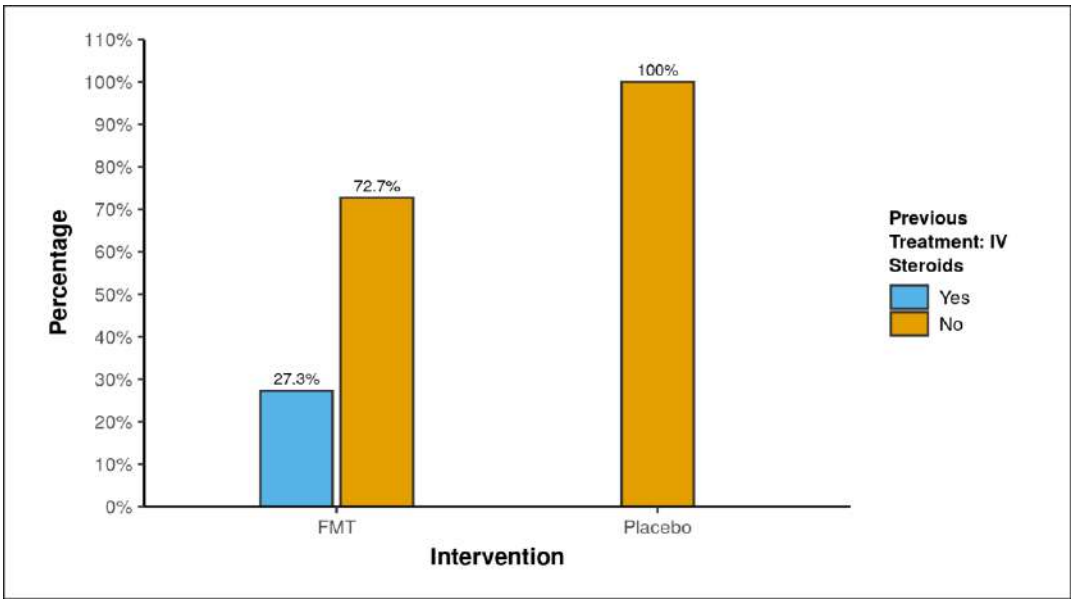


Figure 11: Previous treatment with intravenous steroids

Oral prednisolone had been used previously in 45.5% of the patients in FMT group while 44.4% of the patients in the placebo group reported previous history of oral prednisolone use. There was no significant difference between the two groups regarding oral prednisolone use in the past. ($\chi 2 = 0.002$, $p = 1.000$).

Table 15: Previous treatment with oral prednisolone

Previous Treatment: Oral Prednisolone	Intervention			Fisher's Exact Test	
	FMT	Placebo	Total	χ2	P Value
Yes	5 (45.5%)	4 (44.4%)	9 (45.0%)	0.002	1.000
No	6 (54.5%)	5 (55.6%)	11 (55.0%)		
Total	11 (100.0%)	9 (100.0%)	20 (100.0%)		

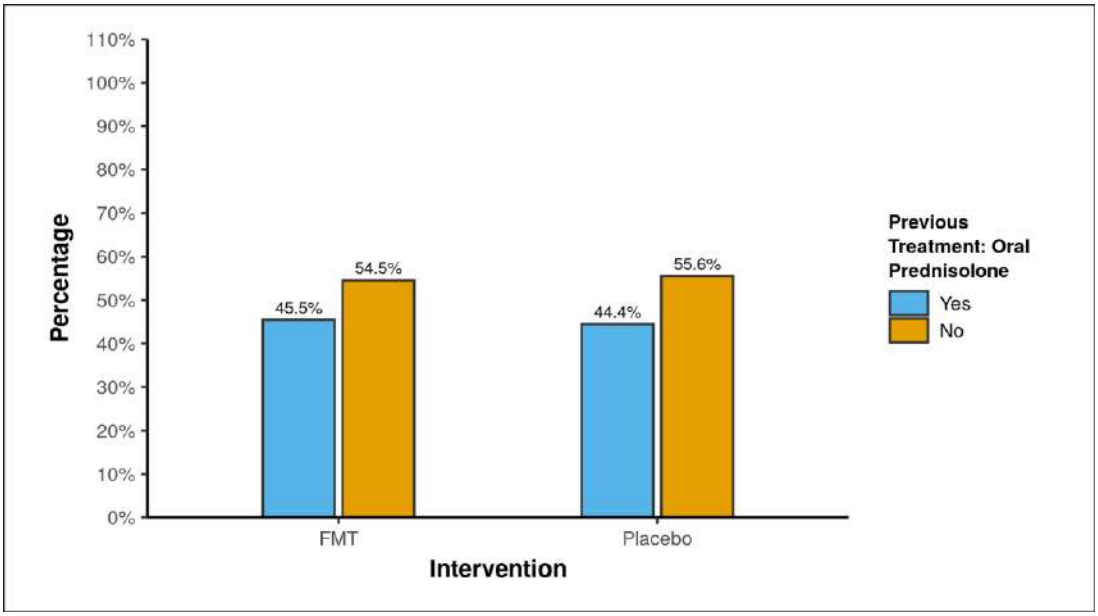


Figure 12: Previous treatment with oral prednisolone

Oral budesonide was used previously in 45.5% of patients in the FMT group and 11.1 % in the placebo group. The difference between the two groups in terms of previous treatment with oral budesonide was not statistically significant. ($\chi 2 = 2.780$, $p = 0.157$).

Table 16: Previous treatment with oral budesonide

Previous Treatment: Oral Budesonide	Intervention			Fisher's Exact Test	
	FMT	Placebo	Total	χ2	P Value
Yes	5 (45.5%)	1 (11.1%)	6 (30.0%)	2.780	0.157
No	6 (54.5%)	8 (88.9%)	14 (70.0%)		
Total	11 (100.0%)	9 (100.0%)	20 (100.0%)		

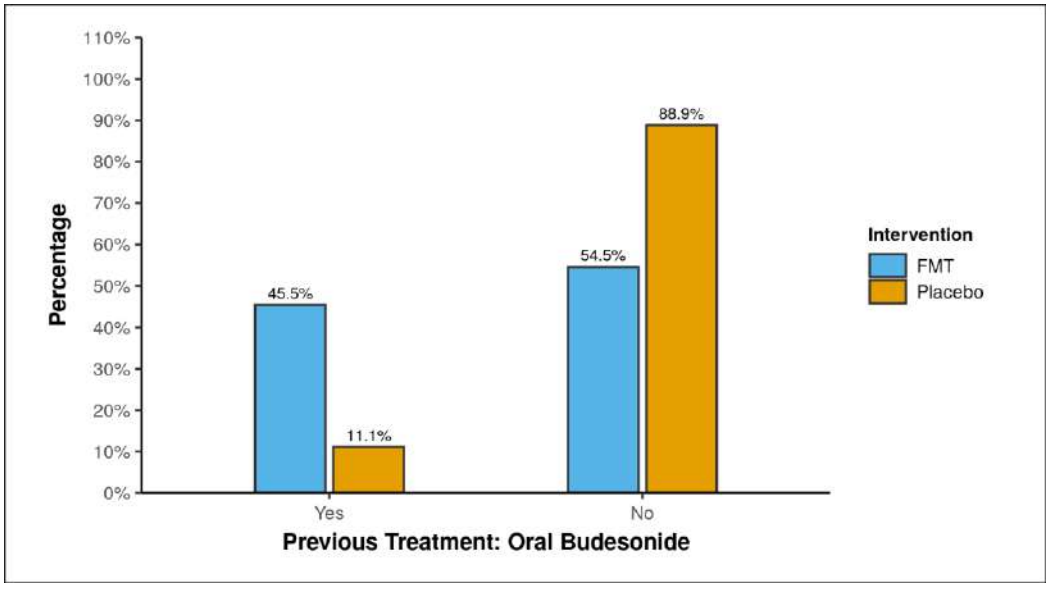


Figure 13: Previous treatment with oral budesonide

All of the participants in the FMT group had received 5-ASA previously while in the placebo group 88.9% had treatment history with 5-ASA. There was no significant difference between the two groups in terms treatment history with 5-ASA. ($\chi 2 = 1.287$, $p = 0.450$).

Table 17: Previous treatment with oral 5-ASA

Previous Treatment: 5-ASA	Intervention			Fisher's Exact Test	
	FMT	Placebo	Total	χ2	P Value
Yes	11 (100.0%)	8 (88.9%)	19 (95.0%)	1.287	0.450
No	0 (0.0%)	1 (11.1%)	1 (5.0%)		
Total	11 (100.0%)	9 (100.0%)	20 (100.0%)		

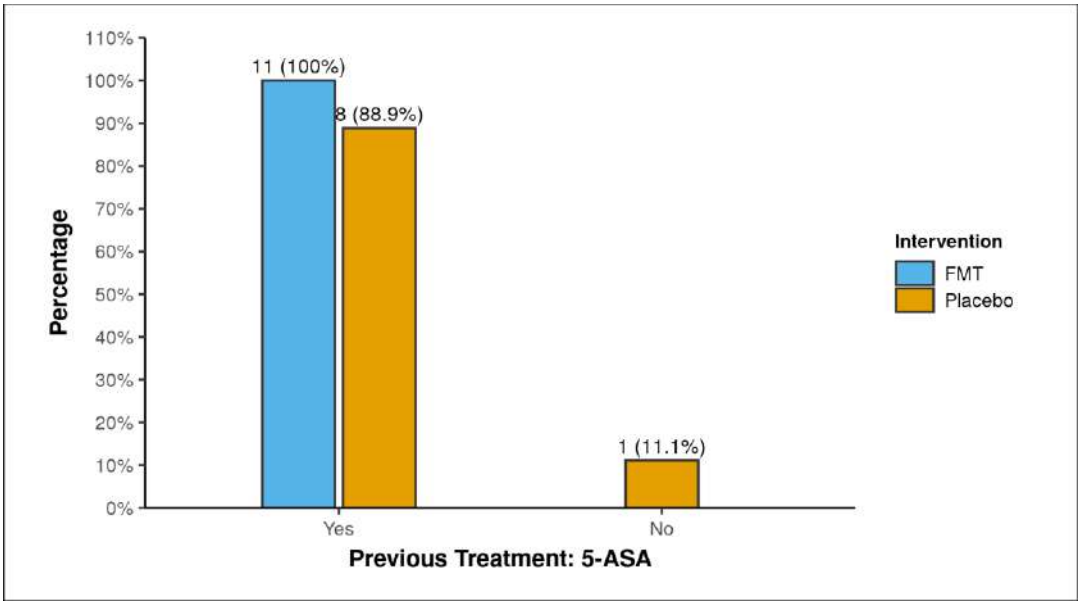


Figure 14: Previous treatment with oral 5-ASA

AZA/ 6- MP was used previously in 27.3% of the participants in the FMT group while 44.4% of the participants in the placebo group had history of AZA/ 6- MP use. There was no significant difference between the two groups in terms of previous treatment with AZA/ 6- MP ($\chi 2$ = 0.642, p = 0.642).

Table 18: Previous treatment with AZA/6-MP

Previous Treatment: AZA/ 6- MP	Intervention			Fisher's Exact Test	
	FMT	Placebo	Total	χ2	P Value
Yes	3 (27.3%)	4 (44.4%)	7 (35.0%)	0.642	0.642
No	8 (72.7%)	5 (55.6%)	13 (65.0%)		
Total	11 (100.0%)	9 (100.0%)	20 (100.0%)		

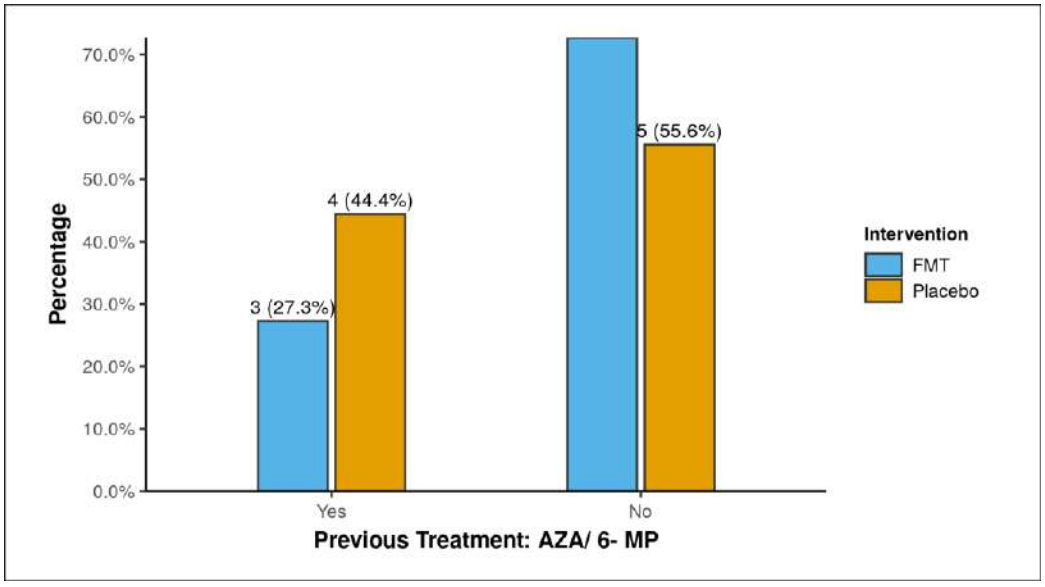


Figure 15: Previous treatment with AZA/6-MP

27.3% of the participants in the FMT group had treatment history with tofacitinib while in the placebo group none of the participants had history of tofacitinib use. The difference between the two groups in terms of previous treatment with tofacitinib was not statistically significant. ($\chi 2 = 2.888$, $p = 0.218$).

Table 19: Previous treatment with tofacitinib

Previous Treatment: TOFA	Intervention			Fisher's Exact Test	
	FMT	Placebo	Total	χ2	P Value
Yes	3 (27.3%)	0 (0.0%)	3 (15.0%)	2.888	0.218
No	8 (72.7%)	9 (100.0%)	17 (85.0%)		
Total	11 (100.0%)	9 (100.0%)	20 (100.0%)		

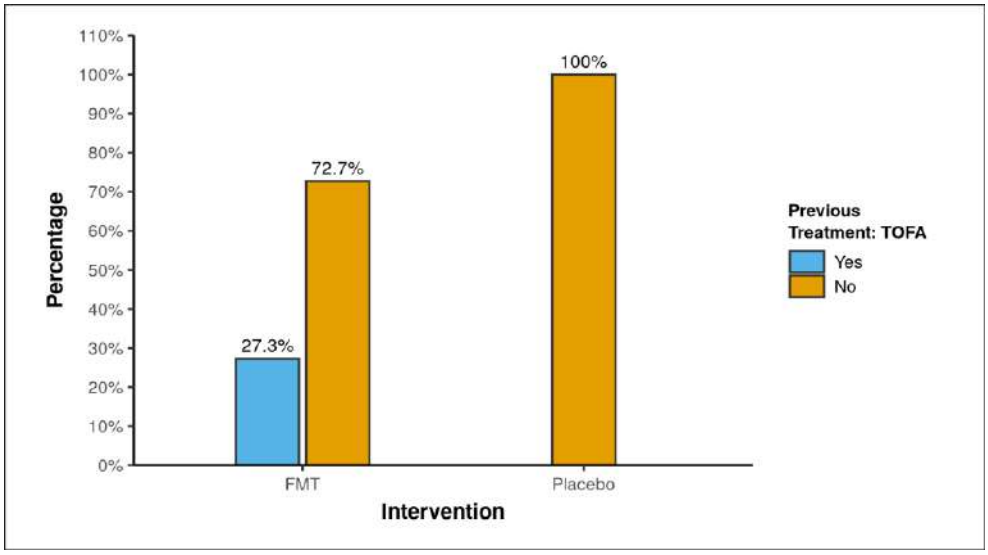


Figure 16: Previous treatment with tofacitinib

None of the patients in the FMT group had history of adalimumab use while one patient in the placebo group had previous history of treatment with adalimumab while There was no significant difference between the two groups in terms of previous treatment with adalimumab ($\chi 2 = 1.287$, $p = 0.450$). None of the patients reported previous treatment history with Infliximab, Vedolizumab and methotrexate.

Table 20: **Previous treatment with adalimumab**

Previous Treatment: ADA	Intervention			Fisher's Exact Test	
	FMT	Placebo	Total	χ2	P Value
Present	0 (0.0%)	1 (11.1%)	1 (5.0%)	1.287	0.450
Absent	11 (100.0%)	8 (88.9%)	19 (95.0%)		
Total	11 (100.0%)	9 (100.0%)	20 (100.0%)		

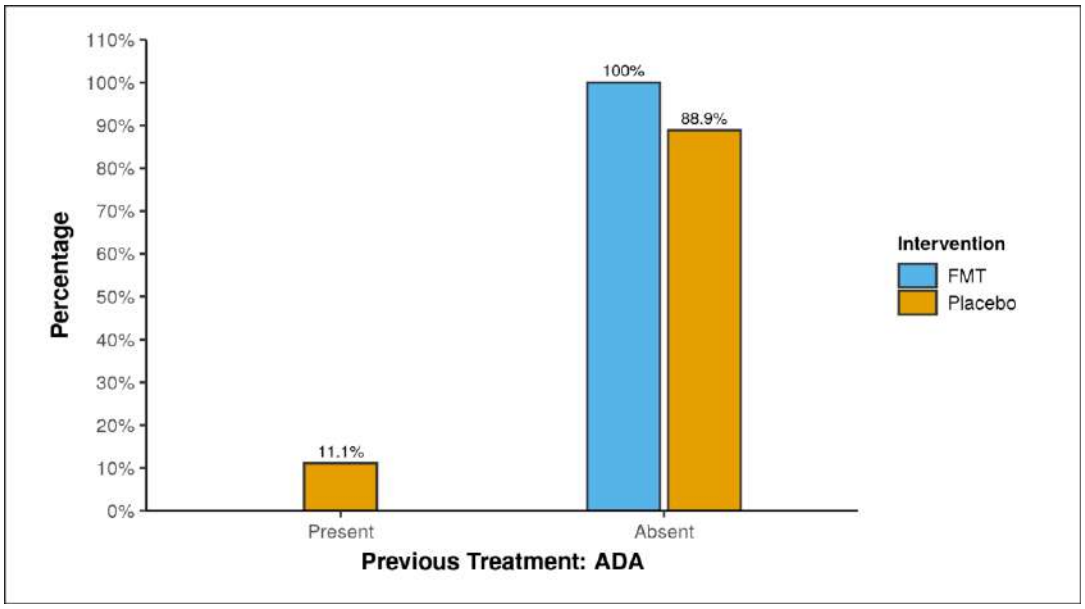


Figure 17: Previous treatment with adalimumab

Baseline investigations

The mean hemoglobin at 1st visit in the FMT group was 10.3±1.82 g/dL. The mean hemoglobin at 1st visit in the placebo group was 9.56±2.14 g/dL. The hemoglobin (g/dL) at 1st visit in the FMT group ranged from 6.4 - 13.7. The hemoglobin (g/dL) at 1st Visit in the placebo group ranged from 6.5 - 14.2. No significant difference was seen between the two groups in terms of baseline hemoglobin values (p = 0.196).

Table 21: Mean hemoglobin at baseline in two groups

Hemoglobin (g/dL) (1st Visit)	Intervention		Wilcoxon-Mann-Whitney U Test	
	FMT	Placebo	W	p value
Mean (SD)	10.32 (1.82)	9.56 (2.14)	67.000	0.196
Median (IQR)	10.6 (9.8-11.05)	9.2 (8.7-10)		
Min - Max	6.4 - 13.7	6.5 - 14.2		

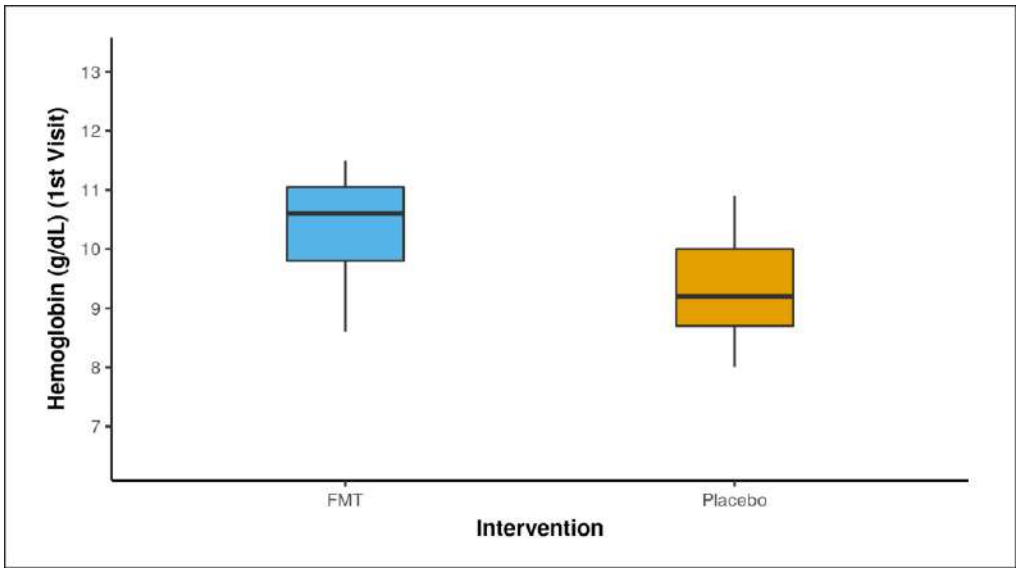


Figure 18: Hemoglobin at baseline in two groups

The mean PCV at 1st visit in the FMT group was 32.17±6.03 %. The mean PCV at 1st visit in the placebo group was 30.08±5.19 %. No significant difference was seen between the two groups in terms of PCV at 1st visit (p = 0.223).

Table 22: PCV at baseline in two groups

PCV (%) (1st Visit)	Intervention		Wilcoxon-Mann-Whitney U Test	
	FMT	Placebo	W	p value
Mean (SD)	32.17 (6.03)	30.08 (5.19)	66.000	0.223
Median (IQR)	32.7 (30.85-35.5)	27.7 (27-31.1)		
Min - Max	18 - 41	24 - 41		

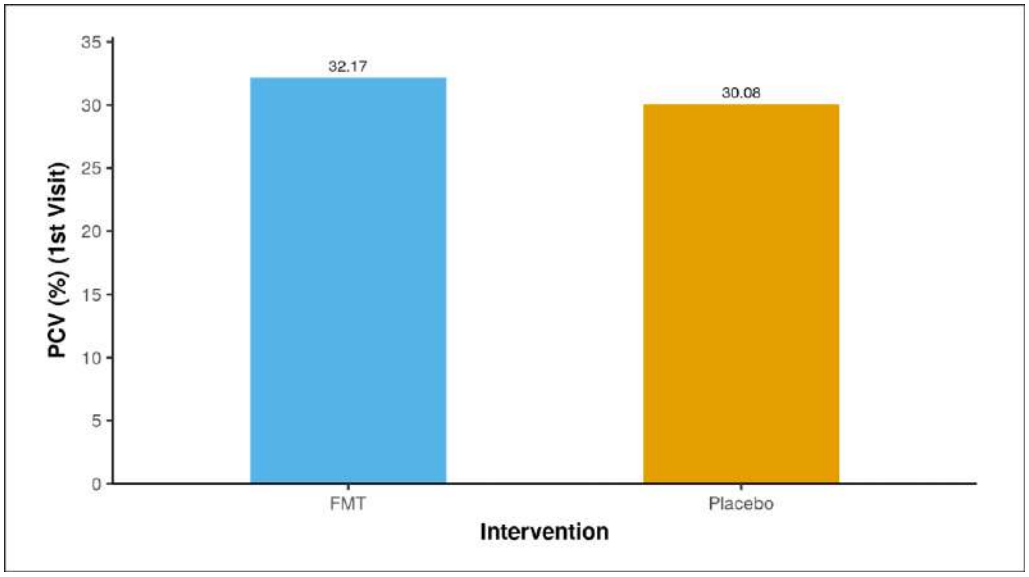


Figure 19: Baseline PCV among two groups

The mean TLC in the FMT group at 1st visit was 7463.64 ±2010.11 /mm³. The mean TLC at 1st visit in the placebo group was 6933.33±1882.82 /mm³. No significant difference was seen between the two groups in terms of TLC counts at 1st visit. (p = 0.543).

The mean platelet counts at 1st visit in the FMT group was 298.91±130.56 *10³/mm³. The mean platelet counts at 1st visit in the placebo group was 241.44±95.80 *10³/mm³. No significant difference was seen between the two groups in terms of platelet count at 1st visit) (p = 0.447).

The mean AST at 1st visit in the FMT group was 43.00±49.36 U/L. The mean AST at 1st visit in the placebo group was 26.44±11.18 U/L. No significant difference was seen between the two groups in terms of AST at 1st visit. (p = 0.790).

The mean ALT at 1st visit in the FMT group was 61.18±113.91 U/L. The mean ALT at 1st visit in the placebo group was 28.33±16.63 U/L. No significant difference was seen between the two groups in terms of ALT at 1st visit (p = 0.879).

The mean ALP at 1st visit in the FMT group was 96.91±19.21 U/L. The mean ALP at 1st visit in the placebo group was 87.44±20.79 U/L. No significant difference was seen between the two groups in terms of ALP at 1st visit (p = 0.342).

The mean serum creatinine at 1st visit in the FMT group was 0.82±0.15 mg/dL. The mean serum creatinine at 1st visit in the placebo group was 0.83±0.24 mg/dL. No significant difference was seen between the two groups in terms of serum creatinine at 1st visit (p = 0.970).

The mean CRP at 1st visit in the FMT group was 15.73±31.29 mg/dL. The mean CRP at 1st Visit in the placebo group was 8.74±10.91 mg/dL. The CRP (mg/L) at 1st visit in the FMT group ranged from 1.6 - 109.2. The CRP at 1st visit in the placebo group ranged from 0.98 - 35.76. There was no significant difference among the two groups in terms of baseline CRP values (p = 0.766).

Figure 19: Baseline CRP among two groups

CRP (mg/dL)	Intervention		Wilcoxon-Mann-Whitney U Test	
	FMT	Placebo	W	p value
Mean (SD)	15.78 (31.29)	8.74 (10.91)	54.000	0.766
Median (IQR)	5.6 (3.08-10.4)	5.82 (3.46-6.8)		
Min - Max	1.6 - 109.2	0.98 - 35.76		

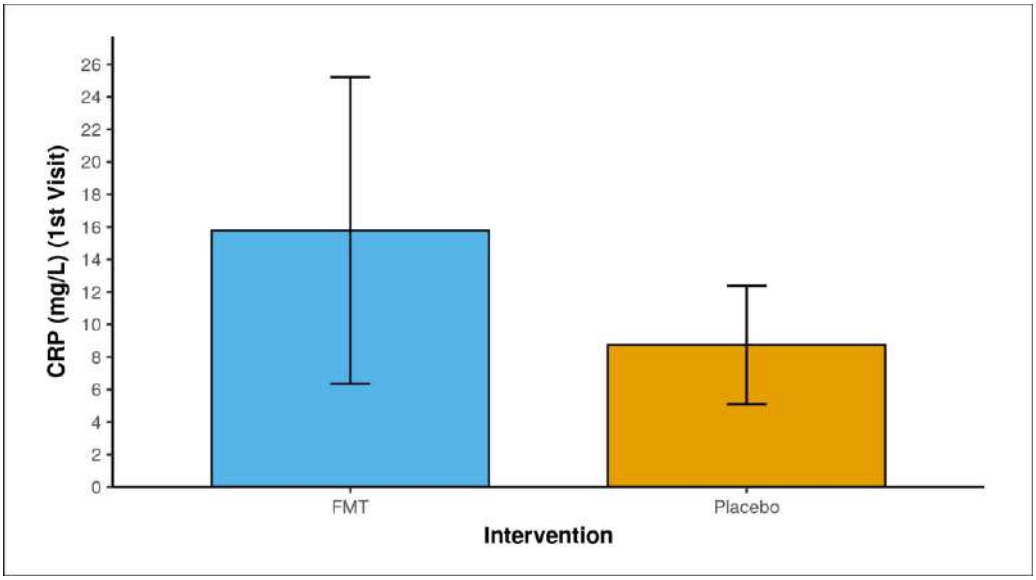


Figure 20: Baseline CRP among two groups

Comparison of baseline fecal calprotectin in two groups

The mean fecal calprotectin at 1st visit in the FMT group was 976.09 ±1117.81 µg/g. The mean fecal calprotectin at 1st visit in the placebo group was 426.44±189.67 µg/g. The fecal calprotectin (µg/g) at 1st visit in the FMT group ranged from 65 - 2870. The fecal calprotectin (µg/g) at 1st visit in the placebo group ranged from 82 - 647. There was no significant difference between the groups in terms of baseline fecal calprotectin values (p = 0.790).

Table 24: Baseline fecal calprotectin among two groups

Fecal Calprotectin (µg/g) (Baseline)	Intervention		Wilcoxon-Mann-Whitney U Test	
	FMT	Placebo	W	p value
Mean (SD)	976.09 (1117.81)	426.44 (189.67)	45.500	0.790
Median (IQR)	361 (190.5-1789.5)	390 (380-614)		
Min - Max	65 - 2870	82 - 647		

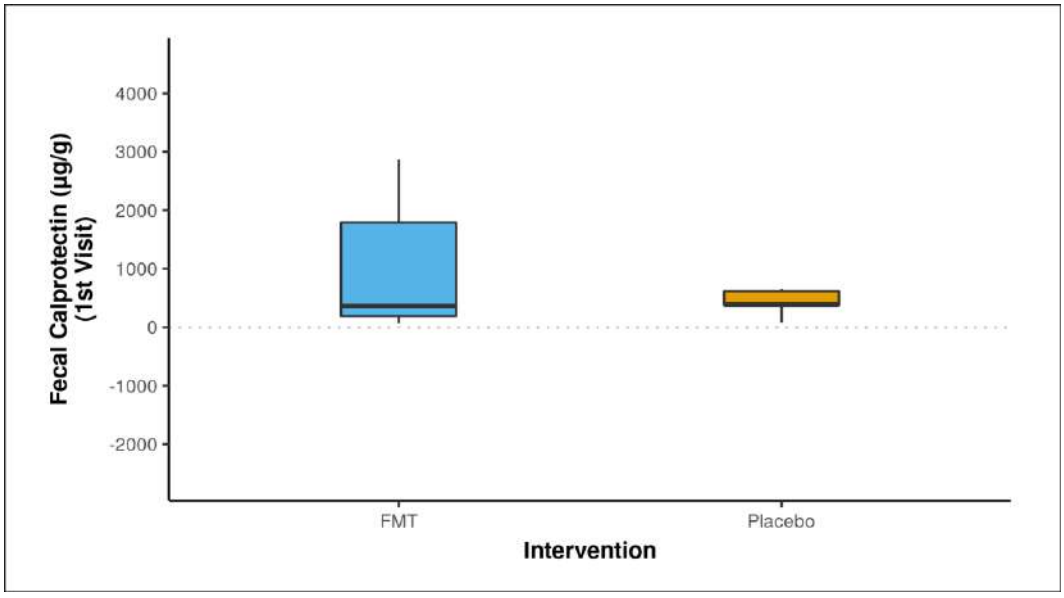


Figure 21: Baseline fecal calprotectin among two groups

FMT procedure

The fecal slurry was infused into the terminal ileum/caecum via colonoscopy. The participants received four sessions of FMT at 0,4,12 and 20 weeks. The mean slurry retention time at 1st visit in the FMT group was 3.82±1.17 hours. The mean slurry retention time at 1st visit in the placebo group was 4.00 ± 0.66 hours. The slurry retention time at 1st visit in the FMT group ranged from 2 – 6 hours. The slurry retention time at 1st visit in the placebo group ranged from 2.5 – 5 hours. There was no significant difference between the two groups in terms of slurry retention time at 1st visit (p = 0.694).

Table 25: Slurry retention time after first FMT

Slurry Retention Time (Hours) (1st Visit)	Intervention		Wilcoxon-Mann-Whitney U Test	
	FMT	Placebo	W	p value
Mean (SD)	3.82 (1.17)	4.00 (0.66)	44.000	0.694
Median (IQR)	4 (3-4.5)	4 (4-4)		
Min - Max	2 - 6	2.5 - 5		

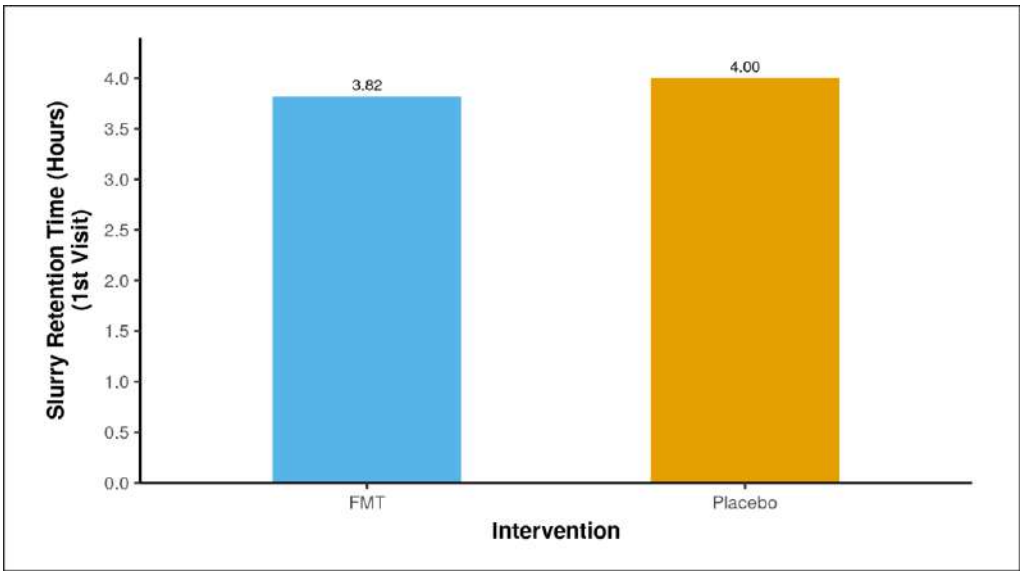


Figure 22: Slurry retention time after first FMT

Change in slurry retention time over study period

Over the time, the mean slurry retention time in FMT group decreased from 2.90 hours at the 2nd visit to a minimum of 2.86 hours at the 3rd visit, and then increased to 2.91 hours at the 4th visit. This change in slurry retention time was not statistically significant (Friedman Test: $\chi 2 = 0.2$, $p = 0.895$). In placebo group, the mean slurry retention time increased from 2.83 hours at the 2nd visit to a maximum of 3.06 hours at the 3rd visit, and then decreased to 2.75 hours at the 4th visit. This change was not statistically significant (Friedman Test: $\chi 2 = 2.4$, $p = 0.296$). There was no significant difference in the trend of slurry retention time over time between the two groups ($p = 0.219$).

Table 26: Comparison of change in slurry retention time among two groups

Slurry Retention Time	Intervention		P value for comparison of the two groups at each of the timepoints (Wilcoxon-Mann-Whitney Test)
	FMT	Placebo	
	Mean (SD)	Mean (SD)	
2nd Visit	2.90 (0.81)	2.83 (0.75)	0.798
3rd Visit	2.86 (0.67)	3.06 (0.50)	0.957
4th Visit	2.91 (0.86)	2.75 (0.38)	0.325
P Value for change in Slurry Retention Time over time within each group (Friedman Test)	0.895	0.296	
Overall P Value for comparison of change in Slurry Retention Time over time between the two groups (Generalized Estimating Equations)	0.219		

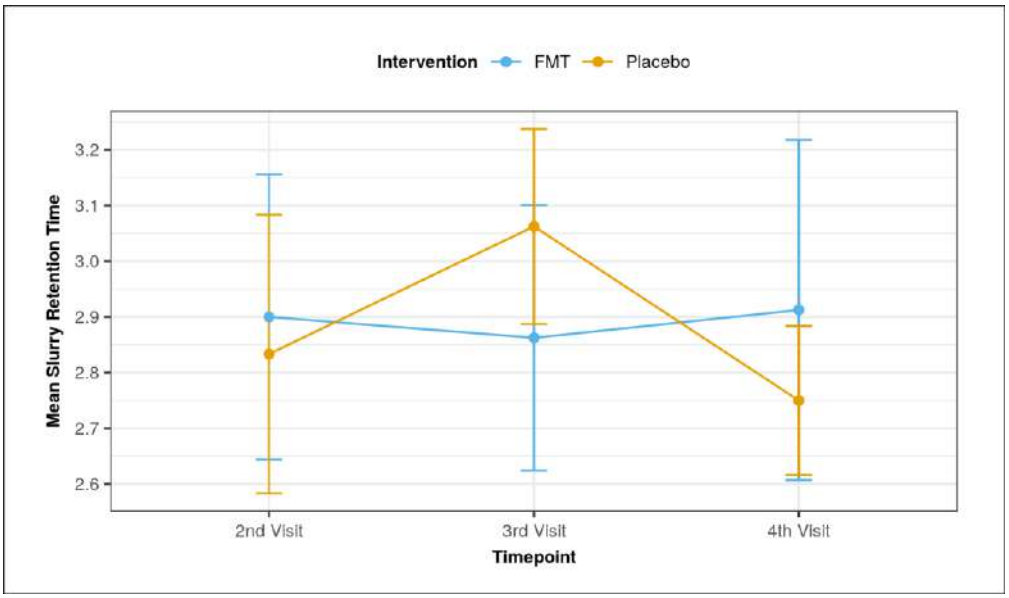


Figure 23: Change in Slurry Retention Time Over Time

Change in clinical disease activity over time

Over the study period, the mean CDAI in FMT group decreased from a maximum of 172.27 at 1st visit to a minimum of 78.62 at the 4th visit. The decline in CDAI was statistically significant (Friedman Test: $\chi 2 = 16.4$, $p = <0.001$). In placebo group, the mean CDAI decreased from a maximum of 182.56 at the 1st visit to a minimum of 137.62 at the 4th visit. This change was statistically significant (Friedman Test: $\chi 2 = 10.1$, $p = 0.018$). The two groups differed significantly in terms of CDAI at 3rd and 4th visit. There was a significant difference in the trend of CDAI over time between the two groups ($p = 0.048$).

Table 27: Comparison of change in CDAI score among two groups

CDAI	Intervention		P value for comparison of the two groups at each of the timepoints (Wilcoxon-Mann-Whitney Test)
	FMT	Placebo	
	Mean (SD)	Mean (SD)	
1st Visit	172.27 (36.66)	182.56 (33.67)	0.517
2nd Visit	133.60 (78.21)	142.56 (39.93)	0.078
3rd Visit	105.12 (26.11)	164.00 (56.74)	0.038
4th Visit	78.62 (35.58)	137.62 (44.26)	0.016
P Value for change in CDAI over time within each group (Friedman Test)	<0.001	0.018	
Overall P Value for comparison of change in CDAI over time between the two groups (Generalized Estimating Equations)	0.048		

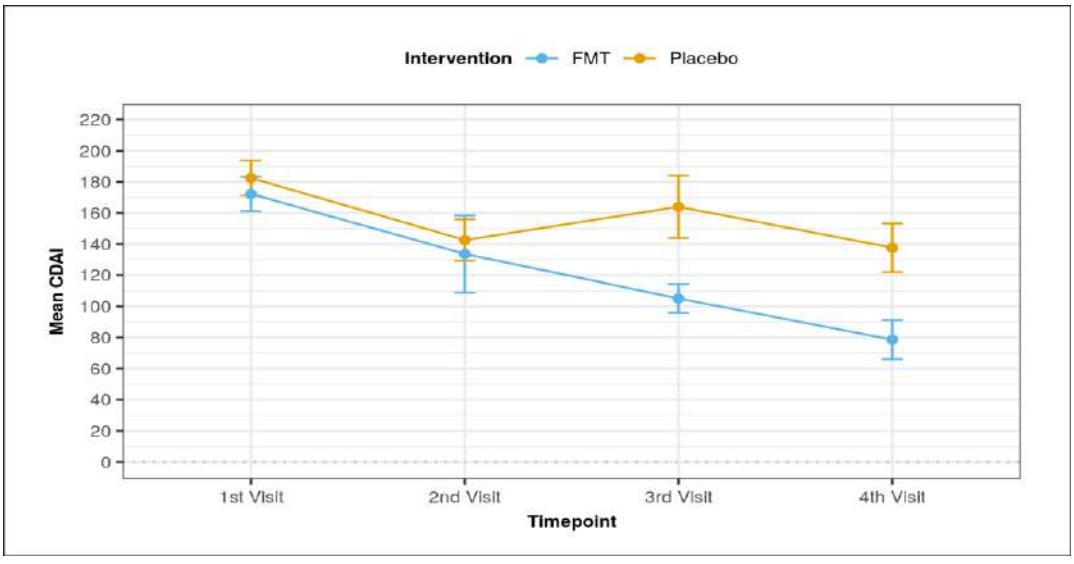


Figure 24: Change in CDAI Over Time

Over the study period, the mean HBI in FMT group decreased from a maximum of 3.18 at the 1st visit to a minimum of 0.75 at the 3rd visit and then increased to 1.62 at the 4th visit. This decline was statistically significant (Friedman Test: $\chi 2 = 13.1$, $p = 0.004$). In placebo group, the mean HBI decreased from a maximum of 3.89 at the 1st visit to a minimum of 1.89 at the 2nd visit and then increased to 2.12 at the 4th visit. This decline was statistically significant (Friedman Test: $\chi 2 = 10.7$, $p = 0.013$). The two groups differed significantly in terms of HBI at the 3rd Visit. There was a significant difference in the trend of HBI over time between the two groups ($p = 0.042$).

Table 28: Comparison of change in HBI score among two groups

HBI	Intervention		P value for comparison of the two groups at each of the timepoints (Wilcoxon-Mann-Whitney Test)
	FMT	Placebo	
	Mean (SD)	Mean (SD)	
1st Visit	3.18 (2.23)	3.89 (1.69)	0.227
2nd Visit	2.40 (1.71)	1.89 (1.05)	0.583
3rd Visit	0.75 (0.71)	2.50 (1.51)	0.015
4th Visit	1.62 (1.51)	2.12 (0.99)	0.388
P Value for change in HBI over time within each group (Friedman Test)	0.004	0.013	
Overall P Value for comparison of change in HBI over time between the two groups (Generalized Estimating Equations)	0.042		

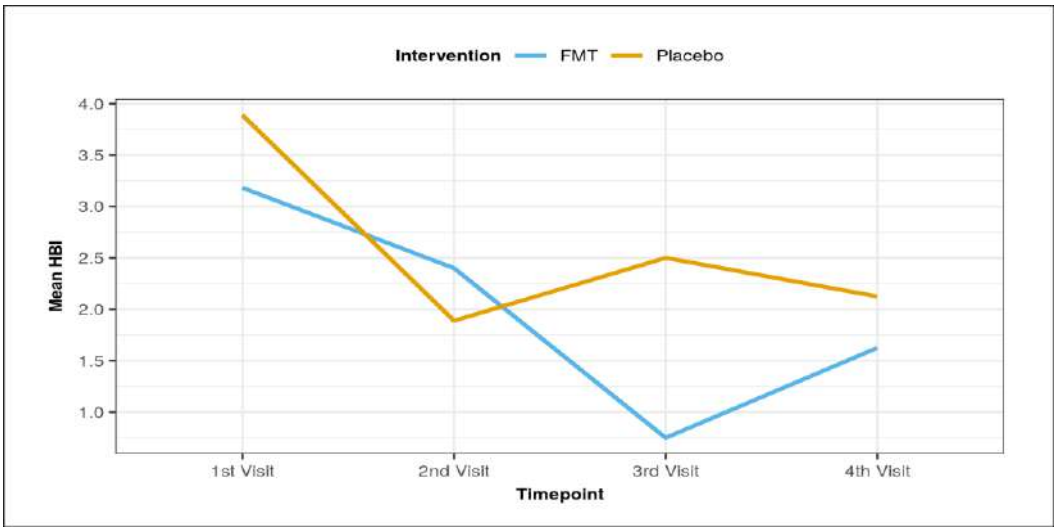


Figure 25: Change in HBI Over Time

Comparison of change in clinical response over time in two groups

10% of patients (n=1) achieved clinical response in both FMT and placebo groups at 2nd visit (p= 1.000). 12.5 % of patients (n=1) had clinical response in FMT group while none of the patients had clinical response in placebo group at 3rd visit. (p=1.000). 50% of patients (n=4) achieved clinical response in FMT group while 12.5% of patients achieved clinical response in placebo group(p=0.282). This shows that the patients in FMT group were more likely to achieve clinical response over time however the difference between two groups was not statistically significant.

Table 29: Comparison of achievement of clinical response among two groups

Clinical Response	Intervention		X^2	p value	Cramer's V
	FMT	Placebo			
2nd Visit	1 (10.0%)	1 (11.1%)	0.000	1.000	0.000
3rd Visit	1 (12.5%)	0 (0.0%)	0.000	1.000	0.000
4th Visit	4 (50.0%)	1 (12.5%)	1.164	0.282	0.322

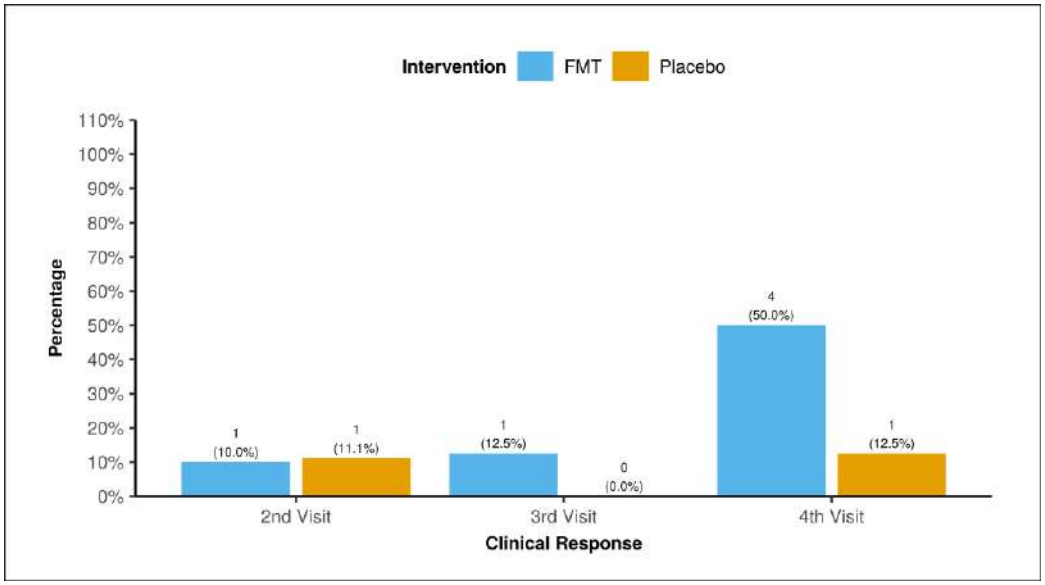


Figure 26: Comparison of clinical response over time

Comparison of clinical remission in two groups

87.5% of the participants(n=7) in both the groups were in clinical remission at 4th visit (at week 20). There was no significant difference between the various groups in terms of distribution of clinical remission ($\chi 2 = 0.000$, $p = 1.000$). Strength of association between the two variables (Bias Corrected Cramer's V) = 0 (Little/No Association)

Table 30: **Comparison of achievement of clinical remission among two groups**

Clinical Remission	Intervention			Fisher's Exact Test	
	FMT	Placebo	Total	χ2	P Value
Yes	7 (87.5%)	7 (87.5%)	14 (87.5%)	0.000	1.000
No	1 (12.5%)	1 (12.5%)	2 (12.5%)		
Total	8 (100.0%)	8 (100.0%)	16 (100.0%)		

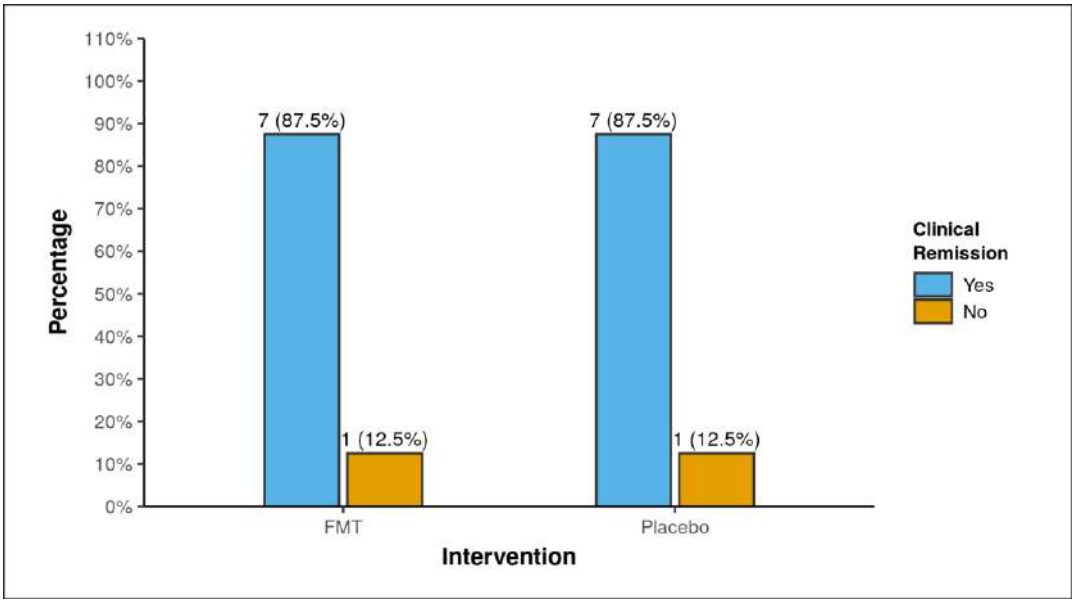


Table 30: **Comparison of achievement of clinical remission among two groups**

Change in endoscopic disease activity over time

Over the study period, the mean SES CD in FMT group decreased from a maximum of 9.73 at 1st visit to a minimum of 4.75 on follow up after completing 4 sessions of FMT. This decline was statistically significant (Friedman Test: $\chi 2 = 15.3$, $p = 0.004$). In placebo group, the mean SES CD decreased from a maximum of 8.56 at the 1st visit timepoint to a minimum of 7.00 at the 2nd visit timepoint and then increased to 8.17 on follow up. This change was not statistically significant (Friedman Test: $\chi 2 = 4.7$, $p = 0.325$). There was a significant difference in the trend of SES CD over time between the two groups ($p = 0.002$).

Table 31: Comparison of change in SES CD over time among two groups

SES CD	Intervention		P value for comparison of the two groups at each of the timepoints (Wilcoxon-Mann-Whitney Test)
	FMT	Placebo	
	Mean (SD)	Mean (SD)	
1st Visit	9.73 (5.98)	8.56 (4.69)	0.647
2nd Visit	9.20 (7.35)	7.00 (3.74)	0.712
3rd Visit	7.50 (6.16)	7.00 (3.16)	0.874
4th Visit	6.62 (5.90)	8.12 (3.68)	0.427
P Value for change in SES CD over time within each group (Friedman Test)	0.004	0.325	
Overall P Value for comparison of change in SES CD over time between the two groups (Generalized Estimating Equations)	0.002		

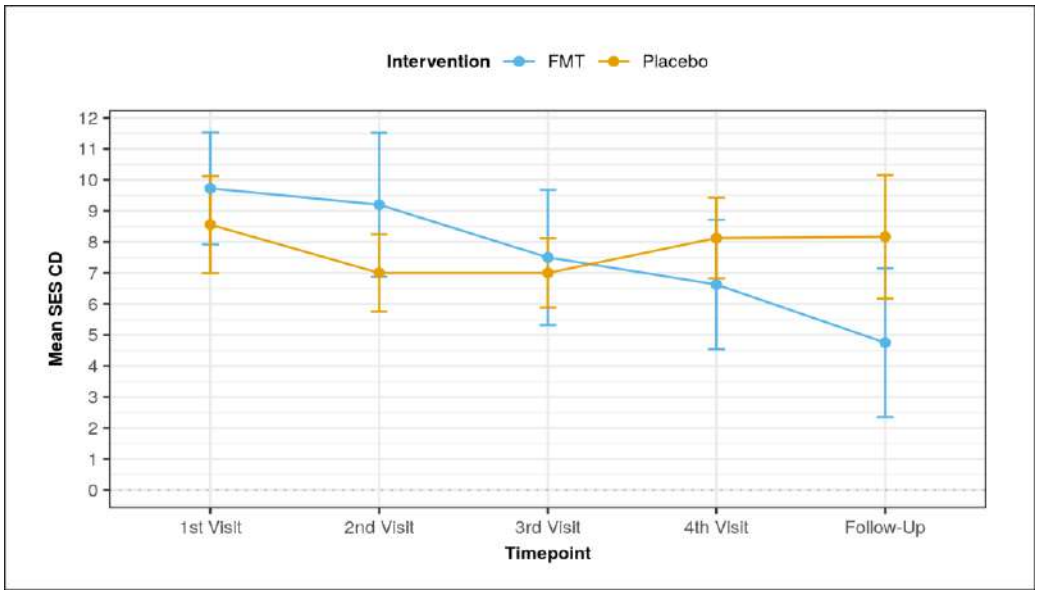


Figure 28: Comparison of SES CD over time

No statistically significant difference was seen in SES CD score between any of the timepoints as compared to baseline SES CD in both placebo and FMT groups. No significant difference was noted between the two groups in terms of percent change in SES CD from the 1st visit to any of the follow-up timepoints.

Table 32: Percent Change in SES CD from 1st Visit to Follow-up Timepoints

Timepoint Comparison	Percent Change in SES CD from 1st Visit to Follow-up Timepoints				P-Value for Comparison of the two Groups in Terms of Difference of SES CD from 1st Visit to Follow-up Timepoints
	Intervention: FMT		Intervention: Placebo		
	Mean (SD) of Percent Change	P Value of Change Within Group	Mean (SD) of Percent Change	P Value of Change Within Group	
2nd Visit - 1st Visit	-10.1% (39.3)	0.990	-17.4% (18.2)	0.665	0.721
3rd Visit - 1st Visit	-13.6% (36.8)	1.000	-21.9% (21.5)	0.297	0.561
4th Visit - 1st Visit	-35.5% (34.2)	1.000	-8.8% (22.7)	1.000	0.111
Follow-Up - 1st Visit	-64.5% (40.8)	0.818	-20.0% (27.0)	0.998	0.099

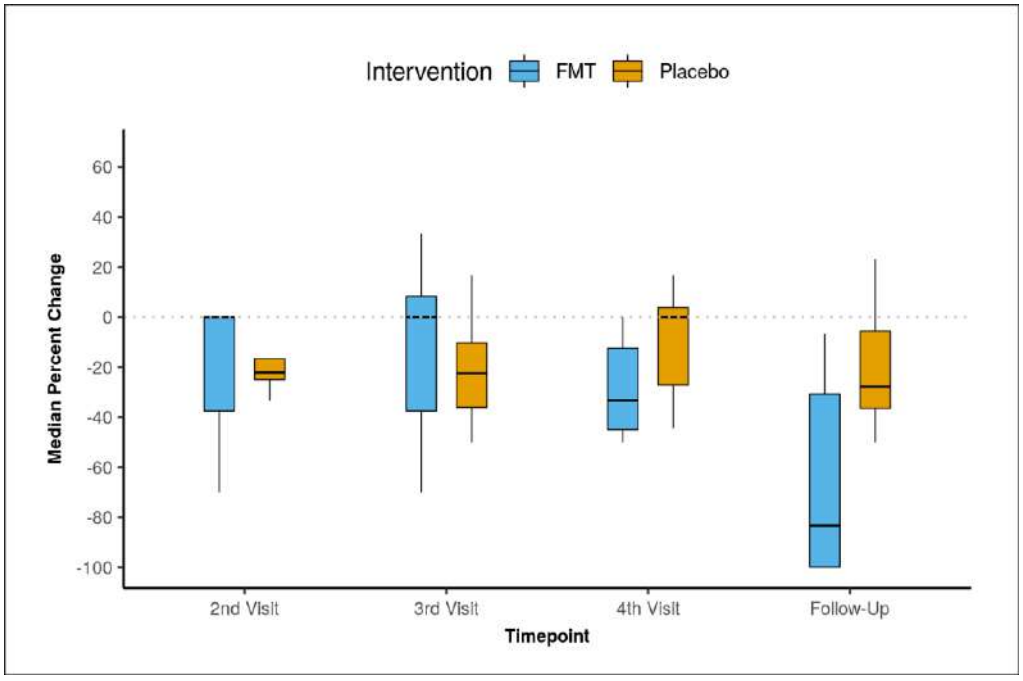


Figure 29: Percent Change in SES CD from 1st Visit to Follow-up Timepoints

Comparison of change in endoscopic response over time in two groups

22.2% of patients (n=2) achieved endoscopic response in FMT group while none of the patients in placebo group showed endoscopic response at 2nd visit (p= 0.471). 28.6% of patients (n=2) had endoscopic response in FMT group while this was seen in 12.5% of the patients in placebo group at 3rd visit. (p=0.569). While the number of patients showing endoscopic response remained same in FMT group (28.6%), none of the patients had endoscopic response in placebo group at 4th visit. (p=0.200). Follow up colonoscopy done at 4 weeks after 4 sessions of FMT showed that 57.5% of patients(n=4) had endoscopic response in FMT group while 16.7% of patients (n=1) had endoscopic response in placebo group(p=0.266).

Table 33: Comparison of achievement of endoscopic response among two groups

Endoscopic Response	Intervention		X^2	p value	Cramer's V
	FMT	Placebo			
2nd Visit	2 (22.2%)	0 (0.0%)	0.562	0.471	0.265
3rd Visit	2 (28.6%)	1 (12.5%)	0.017	0.569	0.000
4th Visit	2 (28.6%)	0 (0.0%)	0.744	0.200	0.335
Follow-Up	4 (57.1%)	1 (16.7%)	0.853	0.266	0.311

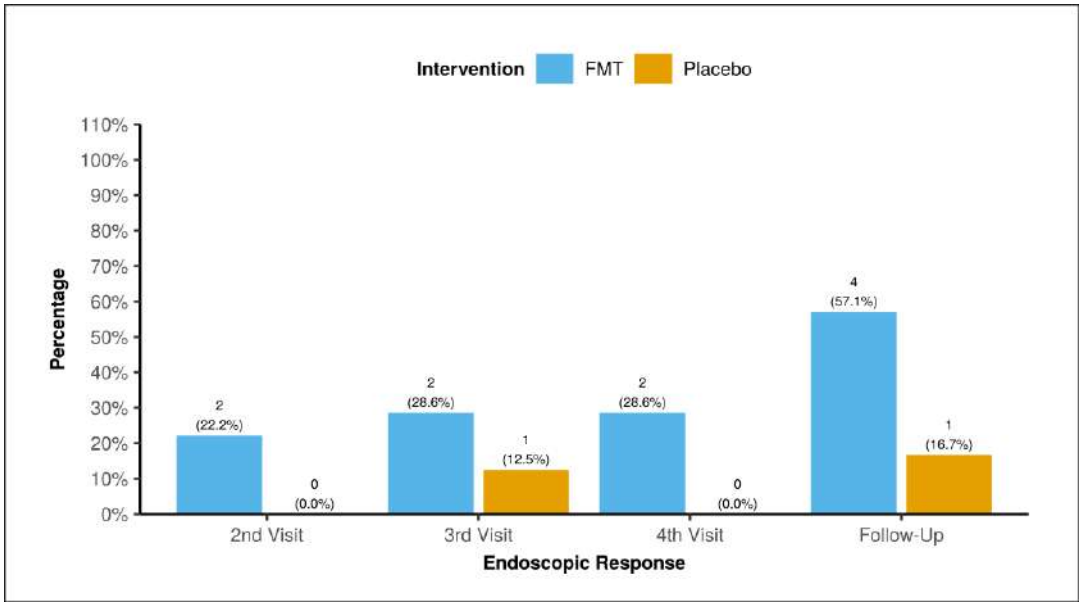


Figure 30: Comparison of Endoscopic Response over time

Comparison of endoscopic remission in two groups

12.5% of the participants in FMT group had endoscopic remission while none of the participants in the placebo had endoscopic remission on colonoscopy done at week 20. There was no significant difference between the two groups in terms of endoscopic remission ($\chi 2 = 5.833$, $p = 0.235$).

Table 34: Comparison of achievement of endoscopic remission among two groups

Endoscopic Remission	Intervention		Fisher's Exact Test	
	FMT	Placebo	χ2	P Value
Yes	1(12.5%)	0 (0.0%)	5.833	0.283
No	7 (87.5%)	8 (100.0%)		
Total	8 (100.0%)	8 (100.0%)		

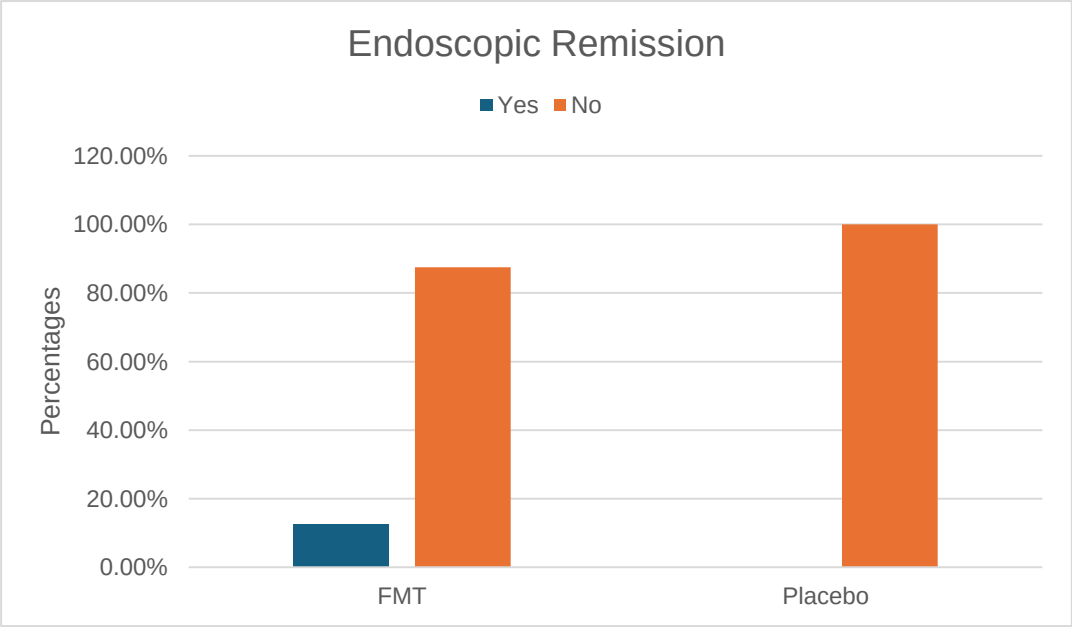


Figure 31: Association between Intervention and Endoscopic Remission

Changes in fecal calprotectin over time in two groups

In FMT group, the mean fecal calprotectin decreased from a maximum of 976.09 µg/g at the 1st visit to a minimum of 217.71 µg/g on follow up (4 weeks after last FMT session). This change was statistically significant (p = 0.047). In placebo group, the mean fecal calprotectin decreased from a maximum of 426.44 µg/g at the 1st visit timepoint to a minimum of 344.50 µg/g on follow up. This change was not statistically significant (p = 0.688). There was a significant difference in the trend of fecal calprotectin over time between the two groups (p = 0.023).

Table 35: Change in Fecal calprotectin after FMT among two groups

Fecal Calprotectin (µg/g)	Intervention		P value for comparison of the two groups at each of the timepoints (Wilcoxon-Mann-Whitney Test)
	FMT	Placebo	
	Mean (SD)	Mean (SD)	
1st Visit	976.09 (1117.81)	426.44 (189.67)	0.790
Follow-Up	217.71 (227.70)	344.50 (296.71)	0.534
P Value for change in Fecal Calprotectin (µg/g) over time within each group (Wilcoxon Test)	0.047	0.688	
Overall P Value for comparison of change in Fecal Calprotectin (µg/g) over time between the two groups (Generalized Estimating Equations)	0.023		

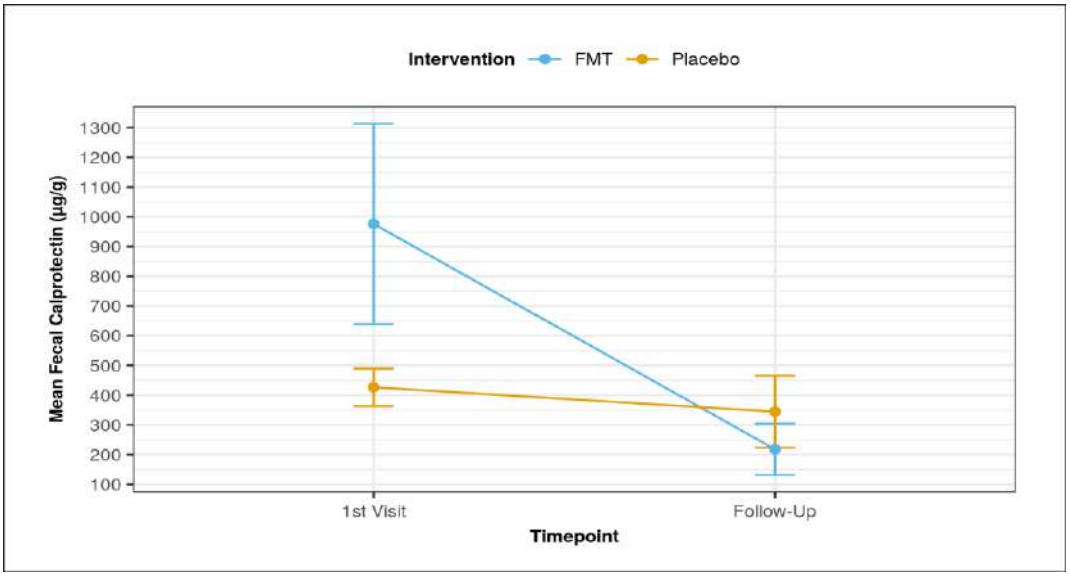


Figure 32: Change in Fecal Calprotectin (µg/g) Over Time

Comparison of change in concomitant medications: oral prednisolone over time in two groups

36.3 % of patients (n=4) in FMT group were on concomitant oral prednisolone as compared to 33.3% of patients (n=3) in placebo group at 1st visit (p=1.000). At 2nd visit timepoint, 30 % of patients (n=3) in FMT group were on concomitant oral prednisolone while this was seen in 11.1 % of patients in placebo group. (p=0.582). None of the patients were on oral prednisolone in both groups at subsequent timepoints. This shows that oral steroids were tapered and stopped in both groups over time.

Table 36: Concomitant oral prednisolone use in two groups

Concomitant Medications: Oral Prednisolone	Intervention		X²	p value	Cramer's V
	FMT	Placebo			
1st Visit	4 (36.4%)	3 (33.3%)	0.000	1.000	0.000
2nd Visit	3 (30.0%)	1 (11.1%)	0.198	0.582	0.000
3rd Visit	0 (0.0%)	0 (0.0%)	Nan	1.000	Nan
4th Visit	0 (0.0%)	0 (0.0%)	Nan	1.000	Nan

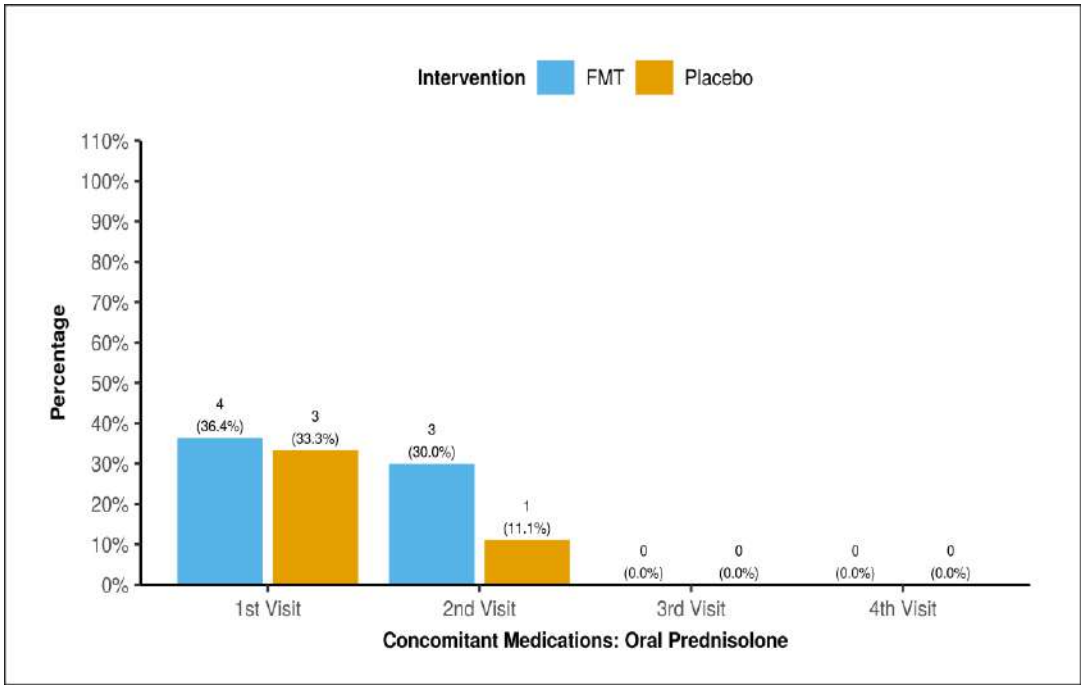


Figure 33: Comparison of change in concomitant oral prednisolone over time

Comparison of change in concomitant medications: oral budesonide over time in two groups

18.2 % of patients (n=2) in FMT group were on concomitant oral budesonide as compared to 22.2 % of patients (n=2) in placebo group at 1st visit (p=1.000). At 2nd visit, 20 % of patients (n=2) in FMT group were on concomitant oral budesonide while this was seen in 22.2 % of patients(n=2) in placebo group. (p=1.000). 12.5 % of patients (n=1) in FMT group were on concomitant oral budesonide as compared to none of the patients in placebo group at 3rd and 4th visit (p=1.000). This shows that oral budesonide was stopped subsequently in placebo group while tapering regimen was followed in FMT group.

Table 37: Concomitant oral budesonide use in two groups

Concomitant Medications: Oral Budesonide	Intervention		X^2	p value	Cramer's V
	FMT	Placebo			
1st Visit	2 (18.2%)	2 (22.2%)	0.000	1.000	0.000
2nd Visit	2 (20.0%)	2 (22.2%)	0.000	1.000	0.000
3rd Visit	1 (12.5%)	0 (0.0%)	0.000	1.000	0.000
4th Visit	1 (12.5%)	0 (0.0%)	0.000	1.000	0.000

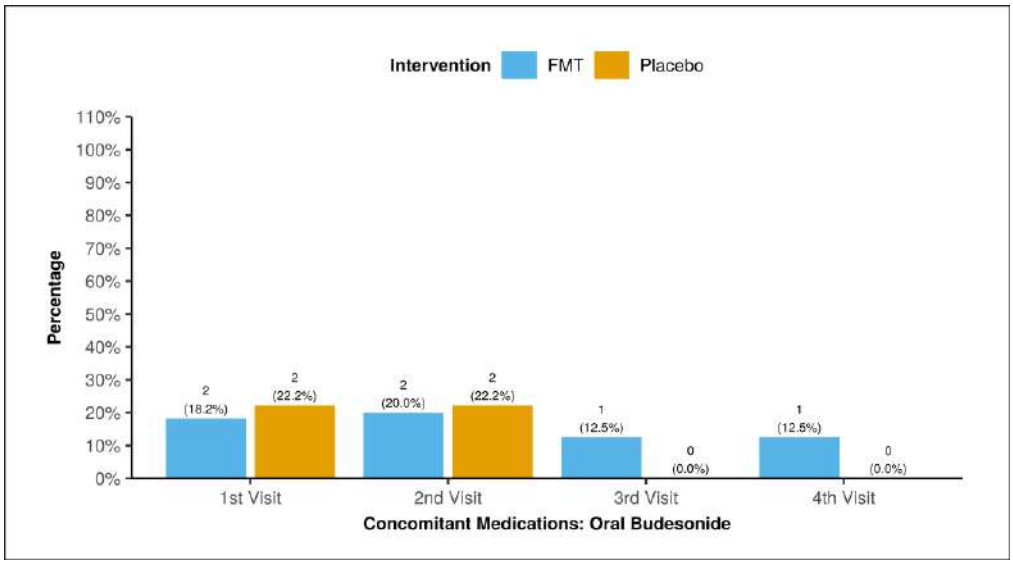


Figure 34: Comparison change in concomitant oral budesonide over time

Comparison of change in concomitant medications: 5-ASA over time in two groups

All of the patients (n=11) in FMT group were on concomitant 5-ASA as compared to 88.9% of patients (n=8) in placebo group at 1st visit (p=0.008). At 2nd visit, all of the patients (n=10) in FMT group were on concomitant 5-ASA while this was seen in 66.7 % of patients(n=6) in placebo group. (p=0.061). All of the patients (n=8) in FMT group were on concomitant 5-ASA as compared to 75% (n=6) and 62.5% (n=5) of the patients in placebo group at 3rd and 4th visit respectively. (p=0.104). This difference was statistically significant only at 1st visit and 2nd visit timepoints.

Table 38: Concomitant oral 5-ASA use in two groups

Concomitant Medications: 5-ASA	Intervention		X^2	p value	Cramer's V
	FMT	Placebo			
1st Visit***	11 (100.0%)	8 (88.9%)	5.669	0.008	0.469
2nd Visit	10 (100.0%)	6 (66.7%)	3.054	0.061	0.352
3rd Visit	8 (100.0%)	6 (75.0%)	1.959	0.104	0.314
4th Visit	8 (100.0%)	5 (62.5%)	1.607	0.123	0.278

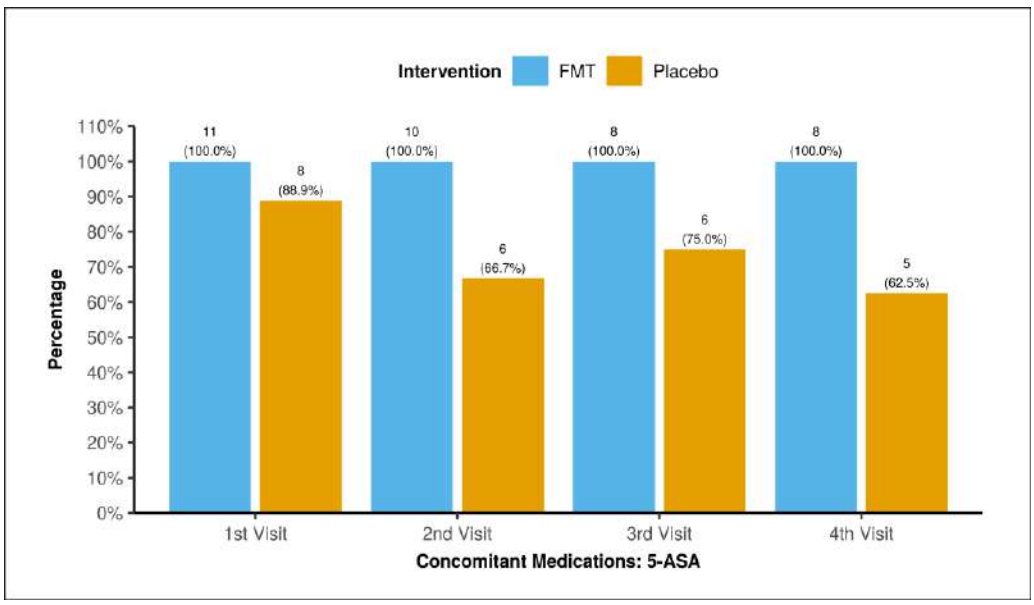


Figure 35: Comparison of change in concomitant 5-ASA over time

Comparison of change in concomitant medications: AZA over time in two groups

27.3% of patients (n=3) in FMT group were on concomitant AZA as compared to 33.3% of patients (n=3) in placebo group at 1st visit (p=1.000). At 2nd visit, 30 % of the patients (n=3) in FMT group were on concomitant AZA while this was seen in 44.4 % of patients(n=4) in placebo group. (p=0.650). 25% of the patients (n=2) in FMT group were on concomitant AZA as compared to 37.5 % (n=3) of the patients in placebo group at both 3rd and 4th visit (p=1.000). The difference was not statistically significant at any timepoints.

Table 39: Concomitant AZA use in two groups

Concomitant Medications: AZA	Intervention		X²	p value	Cramer's V
	FMT	Placebo			
1st Visit	3 (27.3%)	3 (33.3%)	0.000	1.000	0.000
2nd Visit	3 (30.0%)	4 (44.4%)	0.031	0.650	0.000
3rd Visit	2 (25.0%)	3 (37.5%)	0.000	1.000	0.000
4th Visit	2 (25.0%)	3 (37.5%)	0.000	1.000	0.000

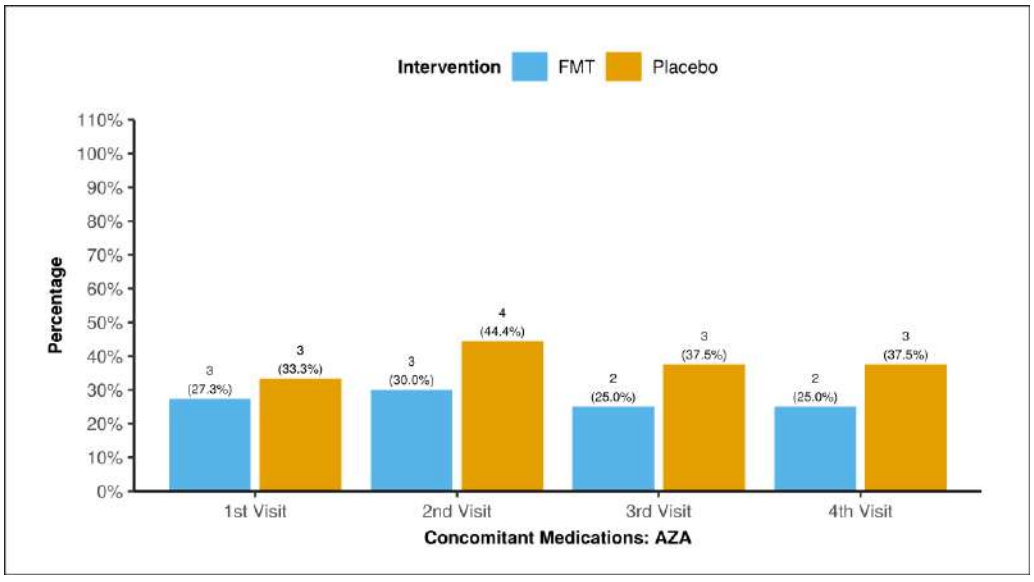


Figure 36: Comparison of change in concomitant medications: AZA over time

Adverse effects in two groups

Most of the adverse events in both groups were mild and self-limited. Mild adverse events included abdominal distension (n=7), Flatulence (n=6), nausea (n=5) and pain abdomen (n=5). Less common adverse events included constipation (n=2) and fever (n=2). One patient had flare of disease activity after 2 sessions of FMT and had change of treatment. In the FMT group**,** Fever (2 vs. 0), Pain abdomen (5 vs. 2), and Nausea (5 vs. 2) occurred more commonly while in placebo group, abdominal distension (5 vs. 2) and flatulence (5 vs. 2) were noted more frequently. The difference was not statistically significant between the two groups. This shows that the adverse events may be related to the procedure itself and not to the FMT.

Table 40: Common side effects after FMT

Symptom	Chi-squared/ Fisher's Exact	p-value
Fever	Fisher's Exact = 2.333	0.126
Pain abdomen	Chi-squared = 2.077	0.149
Nausea	Chi-squared = 2.333	0.126
Abdominal distension	Chi-squared = 2.077	0.149
Flatulence	Chi-squared = 0.049	0.824

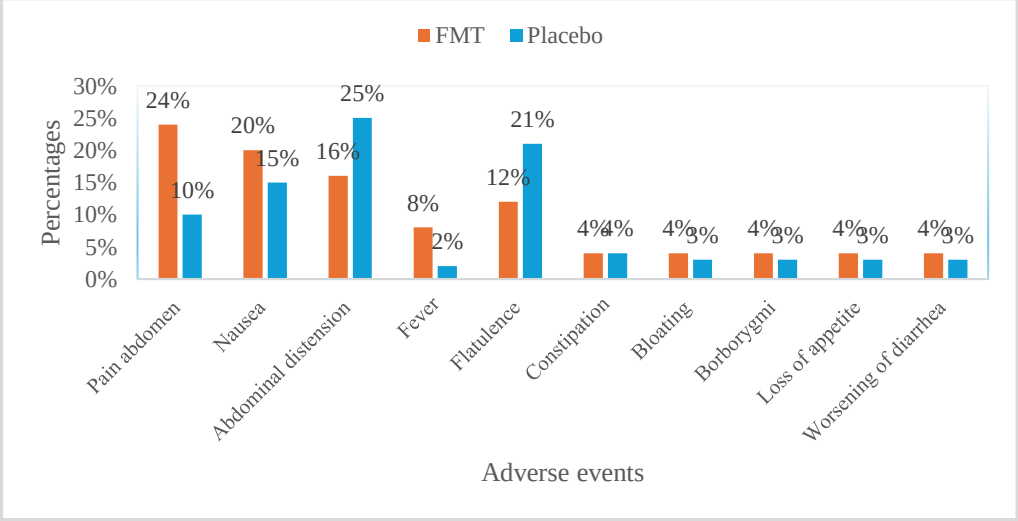


Figure 37: Side effect profile after FMT

DISCUSSION

Crohn's disease is a chronic relapsing-remitting inflammatory bowel disease that can involve any part of the GI tract. The transmural inflammation of gut mucosa results in ulcerations, strictures and fistulae. The disease course can further be complicated by the formation of intraabdominal abscesses and phlegmons. Standard treatment for CD is based on immunosuppressive therapies such as corticosteroids, thiopurines and biologics. These therapies can lead to serious adverse effects in some patients and up to 30 % of patients do not show response to the treatment. Up to 1/3rd of patients require surgery within 10 years of diagnosis.

The pathogenesis of CD is complex and gut dysbiosis plays a key role in addition to aberrant immune response. FMT modulates the gut flora and targets dysbiosis, thus it could be a safe and promising approach in patients with Crohn's disease. The role of FMT in Clostridium difficile colitis and Ulcerative Colitis is well established but data for efficacy in Crohn's disease is sparse. This randomized double-blind placebo controlled trial evaluated the efficacy and safety of FMT in mild to moderate Crohn's disease.

Our study showed that 87.5 % of participants in both the FMT group and placebo group achieved clinical remission at week 20, the difference between the two groups being statistically insignificant.(p=1.000). Clinical response was seen in 10% in FMT group and 11.1% in placebo group at week 4(p=1.000) while it was seen in 50 % in FMT group and 12.5% in placebo group at week 20 (p=0.282). Although the defined outcomes were not met, there was a significant difference in decrease in CDAI score over the study period between two groups (p=0.048). Studies on FMT in CD have shown clinical remission rates ranging between 20-87.5%. The wide variation in the clinical efficacy could be due to difference in methodologies and patient characteristics in different studies.

A proper patient selection is a key to clinical success of FMT. Patients with more severe disease have more gut dysbiosis as compared to patients with milder disease thereby affecting the response to FMT. Most of the patients included in our study had mild disease(mean CDAI=172 in FMT group) which could explain high clinical remission rates seen at week 20. Data on impact of disease severity on outcomes of FMT in Crohn's disease has shown mixed results. Study by Xiang et al showed clinical remission rates and clinical response of 20.1 % and 43.5% respectively at 36 months in patients receiving FMT with better responses seen in patients with milder disease.[43] On the contrary, few studies have shown FMT to be efficacious in patients with severe disease. Study by He Z et al which included patients with penetrating disease reported sustained clinical remission rates of 52% at 3 months.[44] Study by Cui et al with refractory CD patients reported clinical remission in 70% and clinical improvement in 80 % by the third month.[25] Study by Li et al included patients with Infliximab failure and showed clinical response in 78.1% and clinical remission in 62.5% of patients at 3 months.[45] Larger trials and metanalysis could help understand and guide patient selection based on the clinical presentation.

The disease phenotype is also influenced by the age at which Crohn's disease is diagnosed. Pediatric onset Crohn's disease follows a more severe disease course and requires more immunosuppressive therapies as compared to adults.[57] All the patients included in our study were aged more than 18 years. Available data on efficacy of FMT in pediatric CD is limited. Study by Suskind et al showed that 55.6% of patients remained in remission without additional medical therapy at six and twelve weeks after a single session of FMT.[24] Another study by Goyal et al showed that 43% had clinical improvement by sixth month after a single session of FMT and 28% of patients were in clinical remission at 6 months.[58] Higher remission rates seen in our study could be attributed to differences in disease progression, immune

system response, or gut microbiota composition between younger and older patients.

The choice of stool sample for FMT can be either fresh sample or frozen sample. Fresh stool sample was used in our study. Cryopreservation can have a significant impact on the diversity of stool microbiota with a decrease in biodiversity with longer preservation times.[59] Despite these changes, studies have shown fresh and frozen stool FMT to be equally effective in Clostridium difficile colitis.[34] A metanalysis by Cheng et al [49] reported that CD patients who received FMT using fresh stool had a significantly higher clinical remission rate (0.73; I^2 = 0%) compared to patients who received frozen stool (0.43, I^2 = 80%) while another metanalysis by Fang et al reported slightly higher efficacy for fresh stool sample with 36% of patients achieving clinical remission with fresh stool FMT and 28% with frozen stool FMT.[3] These findings show that fresh stool sample could be a better choice for FMT in CD patients.

There is no clear consensus on the optimal route of FMT delivery in CD patients. FMT can be given via upper GI route (via esophagogastroduodenoscopy (EGD), nasogastric or nasoenteric tube), lower GI route (via colonoscopy or retention enema) and oral capsule. Inflamed areas of gut have more pronounced dysbiosis as compared to non inflamed areas and therefore targeted delivery of FMT according to disease location could have an impact on clinical efficacy. FMT was delivered via colonoscopy in our study. Studies on FMT in Crohn's disease have been performed using both lower GI and upper GI routes. Study done by Yang et al compared the efficacy of FMT administered through gastroscopy or colonoscopy which showed clinical remission rates of 69.2% in the gastroscopy group and 64.3% in the colonoscopy group, the difference being statistically non significant.[55] Metanalysis done by Fang et al reported the pooled clinical remission rates of 23% for upper GI route and 27% for lower GI route with statistically non significant difference.[3] These findings show that both routes can be equally effective and the choice of route should

be guided by patient preferences and location of disease. More studies are required to demonstrate whether changing the route of FMT delivery could affect clinical efficacy in patients with Crohn's disease.

Mucosal healing is the target of treatment in patients with CD. Endoscopic evaluation in our study was done at the time of FMT by SES CD score. Our study showed that 22.1% of patients in the FMT group had endoscopic response at week 4 which increased to 28.6 % at week 20 and 57.1% at week 24. There was a significant reduction in SES CD score in the FMT group as compared to the placebo group(p=0.002). Regarding endoscopic remission, one patient had endoscopic remission at week 20 in FMT group while none of the patients achieved endoscopic remission in placebo group. (p=0.234). Limited studies have reported endoscopic outcomes after FMT in Crohn's disease. Study by Sokol et al reported a significant reduction in CDEIS score in the FMT group but not in the sham group(p=0.03) Study by Vaughn et al [26] and Vermeire et al [60] evaluated the efficacy of FMT in CD patients showed no significant change in the CDEIS scores in patients before and after FMT. Although more patients in FMT group had endoscopic response as compared to placebo group in our study, the difference between two groups was not statistically significant.

Repeated sessions of FMT have been proven to more efficacious than single FMT for treatment of recurrent CDI[61] and for maintenance of remission in patients with ulcerative colitis.[22] One plausible explanation is that the host intestine may initially exert colonization resistance to the transplanted microbiota but eventually remodeling occurs and a new balance is established between the foreign microbiota and host microbiota. Studies that followed single FMT protocol in CD patients have shown decrease in clinical remission and response rates over time.[25,45,48] Study by Li et al demonstrated that administering a second FMT within four months of the first can help maintain the clinical benefits achieved from the initial treatment.[50] Patients received 4 sessions of FMT in our study. Clinical response

increased from 10 % at week 4 to 50 % at week 20 in FMT group while 12.5% of patients had

clinical response at week 20 in our study(p=0.282). These findings suggest that multiple sessions of FMT may be better in achieving clinical remission and maintenance of clinical response in patients with Crohn's disease.

Our study has shown that FMT is a safe procedure. Most of the adverse effects were transient and required only supportive management. None of the patients reported long term adverse effects (> 1` month). The most common side effects reported were abdominal distension, nausea, pain abdomen and flatulence. No serious adverse effects occurred after FMT. This is consistent with most of the available studies that have shown FMT as a safe procedure with transient mild adverse effects.

Although there was no significant difference in endoscopic response or remission in two groups, a significant reduction was noted in SES CD score(p=0.002) and fecal calprotectin (p= 0.023) from baseline in FMT group as compared to the placebo group. This finding shows that FMT can have an add on effect in reducing mucosal inflammation in addition to conventional immunosuppressive treatment. The limitations of the study are its small sample size. Studies with larger sample size are required to apply these results on CD population. Another limitation is the stool microbiome analysis which was not done in our study which can help us provide more information regarding microbiota species associated with response and remission.

Table 41: Comparison of the studies done on FMT in Crohn's disease

	Study design	Sample size	Disease phenotype	Disease location	Disease severity	Route of FMT	Number of sessions	Type of stool sample used	Concomitant treatment	Outcomes
Our study	RCT	20	B1/B2	L1/L2/L3	Mild/ moderate	Lower GI	4	Fresh	Yes	CR: 87.5% at week 20 Clinical response: 50 % at week 20
Xiang et al	Uncontrolled trial	214	B1/B2/B3/p B1 76% of cases	L1/L2/L3/L4 L3 58% of cases	Mild/ moderate/ severe	Midgut	Median 3.5	Fresh/ frozen	Yes	CR: 20.1 % at 36 months Clinical response:43.7% at 36 months
Sokol et al	RCT	21	B1/B2	L2/L3	Mild/ moderate	Lower GI	Single	Fresh	None	CR: 50% at 24 weeks
He Z et al	Uncontrolled trial	25	B3	L3 76% of cases	Moderate/ Severe	Upper GI	4	Fresh	Yes(5-ASA)	CR: 52% at 3 months 32% at 12 months
Cui et al	Uncontrolled trial	30	B2/B3	L3 63.3 % of cases	Severe	Midgut	Single	Fresh/ frozen	Yes(5-ASA)	CR: 70% at 3 months Clinical response:80% at 3 months
Gutin et al	Uncontrolled trial	10	B1/B2 B1 70 % of cases	L2 70% of cases	Mild/ moderate	Lower GI	Single	Frozen	Yes	Clinical response: 30% at 1 month
Li et al	Retrospective study	69	B1/B2	L1/L2/L3/L4 L3 60.9% of cases	Moderate/ Severe	Upper GI	3	Fresh/ frozen	Yes (5-ASA)	CR: 43.5% Clinical response: 18.8%
Yang et al	RCT	27	B1/B2	L2	Mild/ moderate	Upper GI/ Lower GI	2	Fresh	Yes	CR: 66.7% at week 8 Clinical response: 77.8% at week 8
Li et al	Retrospective study	36	B1/B2/B3/p B2 43.7% of cases	L1/L2/L3/L4 L3 58% of cases	Severe (IFX failed)	Upper GI	Single	Fresh/ frozen	Yes	CR: 62.5% at 3 months Clinical response:78.1 % at 3 months

SUMMARY AND CONCLUSION

- The primary objective of clinical remission at week 20 was achieved in 87.5% of patients in both FMT group and placebo group.(p=1.000) Clinical response at week 20 was seen in 50 % of patients in FMT group and 12.5 % of patients in placebo group. (p=0.282) Decrease in CDAI score over the study period between the two groups was statistically significant. (p=0.048)
- Endoscopic remission at week 20 was seen in 12.5 % of patients in FMT group and none of the patients in placebo group (p=0.234). Endoscopic response at week 20 was seen in 28.6% in FMT group and none in placebo group. (p=0.266). Decrease in SES CD score over the study period between the two groups was statistically significant (p=0.002)
- Significant decrease in fecal calprotectin from baseline (mean of 976.09 to mean of 217 in FMT group, mean of 426.44 to mean of 344.50 in placebo group) was seen between two groups. (p=0.023)
- Most of the adverse events were mild and self-limiting. Most commonly reported adverse events were abdominal distension, nausea, pain abdomen and flatulence. The difference in adverse events in two groups was not statistically significant.

CONCLUSION

- This study shows that FMT could be an effective approach in patients with Crohn's disease with mild to moderate disease activity.
- Our study employed FMT as an add on therapy in addition to the conventional immunosuppressive treatment which aided in mucosal healing as demonstrated by significant changes in SES CD score and fecal calprotectin.
- FMT is safe procedure with most of the patients reporting only mild self-limited adverse effects.
- Studies with larger sample size and uniform methodologies are required to father investigate the efficacy of FMT in CD patients.

BIBLIOGRAPHY

1. Collins M, DeWitt M. Fecal microbiota transplantation in the treatment of Crohn disease. JAAPA. 2020;33:34-7.
2. Khor B, Gardet A, Xavier RJ. Genetics and pathogenesis of inflammatory bowel disease. Nature. 2011;474:307-17.
3. Fang H, Fu L, Wang J. Protocol for Fecal Microbiota Transplantation in Inflammatory Bowel Disease: A Systematic Review and Meta-Analysis. BioMed Research International. 2018;2018:1–11.
4. Gevers D, Kugathasan S, Denson LA, Vázquez-Baeza Y, Van Treuren W, Ren B, et al. The Treatment-Naive Microbiome in New-Onset Crohn's Disease. Cell Host & Microbe. 2014;15:382–92.
5. Pascal V, Pozuelo M, Borruel N, Casellas F, Campos D, Santiago A, et al. A microbial signature for Crohn's disease. Gut. 2017;66:813–22.
6. Matsuoka K, Kanai T. The gut microbiota and inflammatory bowel disease. Semin Immunopathol. 2015;37:47–55.
7. Joossens M, Huys G, Cnockaert M, De Preter V, Verbeke K, Rutgeerts P, et al. Dysbiosis of the faecal microbiota in patients with Crohn's disease and their unaffected relatives. Gut. 2011;60:631–7.
8. Frank DN, Robertson CE, Hamm CM, Kpadeh Z, Zhang T, Chen H, et al. Disease phenotype and genotype are associated with shifts in intestinal-associated microbiota in inflammatory bowel diseases. Inflamm Bowel Dis. 2011;17:179–84.
9. Syal G, Kashani A, Shih DQ. Fecal Microbiota Transplantation in Inflammatory Bowel Disease: A Primer for Internists. The American Journal of Medicine. 2018;131:1017–24.
10. Dey N, Soergel DA, Repo S, Brenner SE. Association of gut microbiota with post-operative clinical course in Crohn's disease. BMC Gastroenterology. 2013;13:131.
11. Buffet-Bataillon S, Bouguen G, Fleury F, Cattoir V, Le Cunff Y. Gut microbiota analysis for prediction of clinical relapse in Crohn's disease. Sci Rep. 2022;12:19929.

12. Bak SH, Choi HH, Lee J, Kim MH, Lee YH, Kim JS, et al. Fecal microbiota transplantation for refractory Crohn's disease. Intest Res. 2017;15:244.
13. Zhang F, Luo W, Shi Y, Fan Z, Ji G. Should we standardize the 1,700-year-old fecal microbiota transplantation? Am J Gastroenterol. 2012;107:1755-6.
14. Eiseman B, Silen W, Bascom GS, Kauvar AJ. Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. Surgery. 1958;44:854–9.
15. Ianiro G, Maida M, Burisch J, Simonelli C, Hold G, Ventimiglia M, et al. Efficacy of different faecal microbiota transplantation protocols for Clostridium difficile infection: A systematic review and meta-analysis. United European Gastroenterol J. 2018;6:1232–44.
16. Hui W, Li T, Liu W, Zhou C, Gao F. Fecal microbiota transplantation for treatment of recurrent C. difficile infection: An updated randomized controlled trial meta-analysis. PLoS One. 2019;14:e0210016.
17. Surawicz CM, Brandt LJ, Binion DG, Ananthakrishnan AN, Curry SR, Gilligan PH, et al. Guidelines for diagnosis, treatment, and prevention of Clostridium difficile infections. Am J Gastroenterol. 2013;108:478–98.
18. Debast SB, Bauer MP, Kuijper EJ, European Society of Clinical Microbiology and Infectious Diseases. European Society of Clinical Microbiology and Infectious Diseases: update of the treatment guidance document for Clostridium difficile infection. Clin Microbiol Infect. 2014;20:1-26.
19. Bennet JD, Brinkman M. Treatment of ulcerative colitis by implantation of normal colonic flora. Lancet. 1989;1:164.
20. Paramsothy S, Kamm MA, Kaakoush NO, Walsh AJ, van den Bogaerde J, Samuel D, et al. Multidonor intensive faecal microbiota transplantation for active ulcerative colitis: a randomised placebo-controlled trial. Lancet. 2017;389:1218–28.
21. Sun D, Li W, Li S, Cen Y, Xu Q, Li Y, et al. Fecal Microbiota Transplantation as a Novel Therapy for Ulcerative Colitis: A Systematic Review and Meta-Analysis. Medicine. 2016;95:e3765.

22. Sood A, Mahajan R, Singh A, Midha V, Mehta V, Narang V, et al. Role of Faecal Microbiota Transplantation for Maintenance of Remission in Patients With Ulcerative Colitis: A Pilot Study. J Crohns Colitis. 2019;13:1311–7.
23. Moayyedi P, Surette MG, Kim PT, Libertucci J, Wolfe M, Onischi C, et al. Fecal Microbiota Transplantation Induces Remission in Patients With Active Ulcerative Colitis in a Randomized Controlled Trial. Gastroenterology. 2015;149:102-109.
24. Suskind DL, Brittnacher MJ, Wahbeh G, Shaffer ML, Hayden HS, Qin X, et al. Fecal Microbial Transplant Effect on Clinical Outcomes and Fecal Microbiome in Active Crohn's Disease: Inflammatory Bowel Diseases. 2015;21:556–63.
25. Cui B, Feng Q, Wang H, Wang M, Peng Z, Li P, et al. Fecal microbiota transplantation through mid-gut for refractory C rohn's disease: Safety, feasibility, and efficacy trial results. J of Gastro and Hepatol. 2015;30:51–8.
26. Vaughn BP, Vatanen T, Allegretti JR, Bai A, Xavier RJ, Korzenik J, et al. Increased Intestinal Microbial Diversity Following Fecal Microbiota Transplant for Active Crohn's Disease: Inflammatory Bowel Diseases. 2016;22:2182-90.
27. Darfeuille-Michaud A, Neut C, Barnich N, Lederman E, Di Martino P, Desreumaux P, et al. Presence of adherent Escherichia coli strains in ileal mucosa of patients with Crohn's disease. Gastroenterology. 1998;115:1405–13.
28. Brandt LJ, Aroniadis OC, Mellow M, Kanatzar A, Kelly C, Park T, et al. Long-term follow-up of colonoscopic fecal microbiota transplant for recurrent Clostridium difficile infection. Am J Gastroenterol. 2012;107:1079-87.
29. Rossen NG, MacDonald JK, de Vries EM, D'Haens GR, de Vos WM, Zoetendal EG, et al. Fecal microbiota transplantation as novel therapy in gastroenterology: A systematic review. World J Gastroenterol. 2015;21:5359–71.

30. Cammarota G, Ianiro G, Tilg H, Rajilić-Stojanović M, Kump P, Satokari R, et al. European consensus conference on faecal microbiota transplantation in clinical practice. Gut. 2017;66:569–80.
31. Weingarden AR, Chen C, Bobr A, Yao D, Lu Y, Nelson VM, et al. Microbiota transplantation restores normal fecal bile acid composition in recurrent Clostridium difficile infection. Am J Physiol Gastrointest Liver Physiol. 2014;306:G310-319.
32. Sokol H, Galperine T, Kapel N, Bourlioux P, Seksik P, Barbut F, et al. Faecal microbiota transplantation in recurrent Clostridium difficile infection: Recommendations from the French Group of Faecal microbiota Transplantation. Dig Liver Dis. 2016;48:242–7.
33. Tang G, Yin W, Liu W. Is frozen fecal microbiota transplantation as effective as fresh fecal microbiota transplantation in patients with recurrent or refractory Clostridium difficile infection: A meta-analysis? Diagn Microbiol Infect Dis. 2017;88:322–9.
34. Lee CH, Steiner T, Petrof EO, Smieja M, Roscoe D, Nematallah A, et al. Frozen vs Fresh Fecal Microbiota Transplantation and Clinical Resolution of Diarrhea in Patients With Recurrent Clostridium difficile Infection: A Randomized Clinical Trial. JAMA. 2016;315:142–9.
35. Vindigni SM, Surawicz CM. Fecal Microbiota Transplantation. Gastroenterol Clin North Am. 2017;46:171–85.
36. Gough E, Shaikh H, Manges AR. Systematic review of intestinal microbiota transplantation (fecal bacteriotherapy) for recurrent Clostridium difficile infection. Clin Infect Dis. 2011;53:994–1002.
37. Blackburn LM, Bales A, Caldwell M, Cordell L, Hamilton S, Kreider H. Fecal microbiota transplantation in patients with cancer undergoing treatment. Clin J Oncol Nurs. 2015;19:111–4.
38. Goldenberg SD, Batra R, Beales I, Digby-Bell JL, Irving PM, Kellingray L, et al. Comparison of Different Strategies for Providing Fecal Microbiota Transplantation to Treat Patients with Recurrent Clostridium difficile Infection in Two English Hospitals: A Review. Infect Dis Ther. 2018;7:71–86.

39. Brandt LJ, Aroniadis OC. An overview of fecal microbiota transplantation: techniques, indications, and outcomes. Gastrointest Endosc. 2013;78:240–9.
40. Persky SE, Brandt LJ. Treatment of recurrent Clostridium difficile-associated diarrhea by administration of donated stool directly through a colonoscope. Am J Gastroenterol. 2000;95:3283–5.
41. Wang JW, Kuo CH, Kuo FC, Wang YK, Hsu WH, Yu FJ, et al. Fecal microbiota transplantation: Review and update. J Formos Med Assoc. 2019;118:S23–31.
42. Peng Z, Xiang J, He Z, Zhang T, Xu L, Cui B, et al. Colonic transendoscopic enteral tubing: A novel way of transplanting fecal microbiota. Endosc Int Open. 2016;4:E610-613.
43. Xiang L, Ding X, Li Q, Wu X, Dai M, Long C, et al. Efficacy of faecal microbiota transplantation in Crohn's disease: a new target treatment? Microbial Biotechnology. 2020;13:760–9.
44. He Z, Li P, Zhu J, Cui B, Xu L, Xiang J, et al. Multiple fresh fecal microbiota transplants induces and maintains clinical remission in Crohn's disease complicated with inflammatory mass. Sci Rep. 2017;7:4753.
45. Li Q, Ding X, Liu Y, Marcella C, Dai M, Zhang T, et al. Fecal Microbiota Transplantation is a Promising Switch Therapy for Patients with Prior Failure of Infliximab in Crohn's Disease. Front Pharmacol. 2021;12:658087.
46. Zhang FM. Fecal microbiota transplantation for severe enterocolonic fistulizing Crohn's disease. WJG. 2013;19:7213.
47. Kao D, Hotte N, Gillevet P, Madsen K. Fecal Microbiota Transplantation Inducing Remission in Crohn's Colitis and the Associated Changes in Fecal Microbial Profile. Journal of Clinical Gastroenterology. 2014;48:625–8.
48. Saint-Antoine IBD Network, Sokol H, Landman C, Seksik P, Berard L, Montil M, et al. Fecal microbiota transplantation to maintain remission in Crohn's disease: a pilot randomized controlled study. Microbiome. 2020;8:12.

49. Cheng F, Huang Z, Wei W, Li Z. Fecal microbiota transplantation for Crohn's disease: a systematic review and meta-analysis. Tech Coloproctol. 2021;25:495–504.
50. Li P, Zhang T, Xiao Y, Tian L, Cui B, Ji G, et al. Timing for the second fecal microbiota transplantation to maintain the long-term benefit from the first treatment for Crohn's disease. Appl Microbiol Biotechnol. 2019;103:349–60.
51. Colman RJ, Rubin DT. Fecal microbiota transplantation as therapy for inflammatory bowel disease: a systematic review and meta-analysis. J Crohns Colitis. 2014;8:1569–81.
52. Wei Y, Zhu W, Gong J, Guo D, Gu L, Li N, et al. Fecal Microbiota Transplantation Improves the Quality of Life in Patients with Inflammatory Bowel Disease. Gastroenterology Research and Practice. 2015;2015:1–5.
53. Gutin L, Piceno Y, Fadrosh D, Lynch K, Zydek M, Kassam Z, et al. Fecal microbiota transplant for Crohn disease: A study evaluating safety, efficacy, and microbiome profile. UEG Journal. 2019;7:807–14.
54. Kong L, Lloyd-Price J, Vatanen T, Seksik P, Beaugerie L, Simon T, et al. Linking Strain Engraftment in Fecal Microbiota Transplantation With Maintenance of Remission in Crohn's Disease. Gastroenterology. 2020;159:2193-2202.e5.
55. Yang Z, Bu C, Yuan W, Shen Z, Quan Y, Wu S, et al. Fecal Microbiota Transplant via Endoscopic Delivering Through Small Intestine and Colon: No Difference for Crohn's Disease. Dig Dis Sci. 2020;65:150–7.
56. Wang H, Cui B, Li Q, Ding X, Li P, Zhang T, et al. The Safety of Fecal Microbiota Transplantation for Crohn's Disease: Findings from A Long-Term Study. Adv Ther. 2018;35:1935–44.
57. Pigneur B, Seksik P, Viola S, Viala J, Beaugerie L, Girardet JP, et al. Natural history of Crohn's disease: comparison between childhood- and adult-onset disease. Inflamm Bowel Dis. 2010;16:953–61.
58. Goyal A, Yeh A, Bush BR, Firek BA, Siebold LM, Rogers MB, et al. Safety, Clinical Response, and Microbiome Findings Following Fecal

Microbiota Transplant in Children With Inflammatory Bowel Disease. Inflammatory Bowel Diseases. 2018;24:410–21.

59. Dorsaz S, Charretier Y, Girard M, Gaïa N, Leo S, Schrenzel J, et al. Changes in Microbiota Profiles After Prolonged Frozen Storage of Stool Suspensions. Front Cell Infect Microbiol. 2020;10:77.
60. Vermeire S, Joossens M, Verbeke K, Wang J, Machiels K, Sabino J, et al. Donor Species Richness Determines Faecal Microbiota Transplantation Success in Inflammatory Bowel Disease. ECCOJC. 2016;10:387–94.
61. Baunwall SMD, Lee MM, Eriksen MK, Mullish BH, Marchesi JR, Dahlerup JF, et al. Faecal microbiota transplantation for recurrent Clostridioides difficile infection: An updated systematic review and meta-analysis. EClinicalMedicine. 2020;29:100642.

S. No.	Name	Mrd	Intervention	Age	Sex	Age at diagnosis	Disease duration	Family history	Appendicectomy	Addictions				Comorbidities					
										Smoking	Alcohol	Opium	IVDU	DM	HTN	CAD	COAD	CVA	Hypothyroidism
1	M	308643	F	56	F	35	20 Y	0	0	0	0	0	0	0	0	0	0	0	0
2	A	181530	F	49	F	30	3 Y	0	0	0	0	0	0	1	0	0	0	0	1
3	IS	1488320	P	40	M	26	14 y	0	0	0	1	0	0	0	0	0	0	0	0
4	S	578170	P	35	F	34	4 mo	0	0	0	0	0	0	0	0	0	0	0	0
5	N	1244876	P	39	M	35	4 Y	0	0	0	0	0	0	0	0	0	0	0	0
6	SK	1475227	P	42	M	40	2 Y	0	0	0	0	0	0	0	0	0	0	0	0
7	S	1486676	P	44	F	43	7 mo	0	0	0	0	0	0	0	0	0	0	0	0
8	SS	1519906	P	48	M	46	2 Y	0	0	0	0	0	0	1	0	0	0	0	0
9	GS	1512954	F	40	M	39	1 Y	0	0	0	1	1	0	0	0	0	0	0	0
10	KK	1249609	F	36	F	33	3 Y	0	0	0	0	0	0	0	0	0	0	0	0
11	PK	1254000	F	25	M	23	2 Y	0	0	0	0	0	0	0	0	0	0	0	0
12	RS	1471103	F	27	M`	26	1 Y	0	0	0	0	0	0	0	0	0	0	0	0
13	SS	1515597	F	38	M	37	1 Y	0	0	0	1	0	0	0	0	0	0	0	0
14	SS	1467639	P	57	M	56	6 mo	0	0	0	0	0	0	0	1	0	0	0	0
15	BK	1560426	P	55	F	54	6 mo	0	0	0	0	0	0	0	0	0	0	0	0
16	BK	1035392	F	43	F	40	2.5 Y	0	0	0	0	0	0	0	0	0	0	0	0
17	SG	963149	F	18	F	15	3 Y	0	0	0	0	0	0	0	0	0	0	0	0
18	A	28215	P	37	F	34	3 Y	0	0	0	0	0	0	0	0	0	0	0	0
19	H	772211	F	21	M	20	1 Y	0	0	0	0	0	0	0	0	0	0	0	0
20	HA	891182	F	41	F	38	3 Y	0	0	0	0	0	0	0	0	0	0	0	0

S. No.		BMI	Disease Location	Disease behaviour	Previous Treatment										1st visit				
	Hyperthyroidism				IV Steroids	Oral Prednisolone	Oral budesonide	5-ASA	AZA/ 6- MP	TOFA	IFX	ADA	VDZ	MTX	Slurry retention time	CDAI	HBI	SES CD	Hb
1	0	25	2	1	0	0	0	1	1	0	0	0	0	0	6	151	6	10	11
2	0	30.1	2	1	1	1	0	1	0	0	0	0	0	0	3	171	3	7	6.4
3	0	22.9	2	1	0	0	0	1	0	0	0	0	0	0	4	153	3	13	8.9
4	0	13.7	1	1	0	0	0	1	0	0	0	0	0	0	4.5	240	6	4	10.9
5	0	19.4	3	2	0	1	0	1	0	1	9	1	0	0	4	157	6	9	14.2
6	0	19	3	1	0	0	0	1	0	0	0	0	0	0	4	152	4	6	8
7	0	26.3	2	1	0	1	0	0	1	0	0	0	0	0	5	151	3	3	9.6
8	0	23.5	1	2	0	0	1	1	1	0	0	0	0	0	4	229	2	8	6.5
9	0	24.2	1	1	0	0	1	1	0	0	0	0	0	0	4.5	138	3	3	11.5
10	0	26.4	1	1	0	0	1	1	0	0	0	0	0	0	4	205	5	6	10.6
11	0	21.1	2	1	1	1	1	1	0	1	0	0	0	0	2	272	8	15	9.6
12	0	17.4	2	1	0	1	0	1	0	1	0	0	0	0	2.5	154	1	6	11.1
13	0	21.1	2	1	1	1	0	1	0	0	0	0	0	0	4	152	1	12	10.8
14	0	17.5	1	2	0	0	0	1	1	0	0	0	0	0	4	195	2	10	10
15	0	20.4	2	1	0	1	0	1	0	0	0	0	0	0	2.5	182	3	18	8.7
16	0	26	3	1	0	0	1	1	0	0	0	0	0	0	4.5	157	2	12	8.6
17	0	21.8	3	1	0	0	0	1	0	1	0	0	0	0	5	152	2	20	10.2
18	0	24.4	1	1	0	1	0	1	1	0	0	0	0	0	4	184	6	6	9.2
19	0	19.7	1	1	0	1	0	1	1	0	0	0	0	0	3	158	2	0	13.7
20	0	19.4	2	1	0	0	1	1	1	0	0	0	0	0	3.5	170	2	16	10

S. No.	Blood parameters									Concomitant medications														
	PCV	TLC	PLT	AST	ALT	ALP	CRP	CREAT	Fecal Cal (Pre)	IV Steroids	Oral Prednisolone	Oral Budesonide	5-ASA	AZA	TOFA	IFX	ADA	VDZ	MTX	Fever	Pain abdomen	Nausea	Vomiting
1	37	6900	408	39	51		2.8	0.91	65	0	0	0	1	1	0	0	0	0	0	0	0	1	0
2	18	9800	301	42	56		8.6	0.86	361	0	1	0	1	0	1	0	0	0	0	0	1	0	0
3	24	10800	270	40	42		3.6	1.2	614	0	0	0	1	0	0	0	0	0	0	0	0	0	0
4	35.3	7100	150	21	15	74	6.8	0.47	82	0	0	1	1	0	0	0	0	0	0	0	0	1	0
5	41	7300	150	38	53	87	5.82	0.68	630	0	0	0	1	0	0	0	1	0	0	0	0	0	0
6	27	6200	132	27	31	72	0.98	0.62	236	0	0	1	1	0	0	0	0	0	0	0	0	0	0
7	31.1	5700	428	21	31	109	35.76	0.81	647	0	1	0	0	1	0	0	0	0	0	0	0	0	0
8	27.7	5600	232	15	13	52	1.24	0.93	380	0	1	0	1	0	0	0	1	0	0	0	0	0	0
9	37	5200	206	33	44	110	15.6	0.96	185	0	0	1	1	0	0	0	0	0	0	0	1	0	0
10	33.7	4200	245	25	15		4.2	0.74	196`	0	0	0	1	0	0	0	0	0	0	0	0	0	0
11	31.1	6300	202	16	18	85	109.2	0.87	2460	0	1	0	1	0	1	0	0	0	0	0	1	0	0
12	34	7200	187	63	27	74	2.46	1.04	2870	0	1	0	1	0	1	0	0	0	0	0	0	0	0
13	32	9700	472	185	401	129	12.2	0.71	380	0	1	0	1	1	0	0	0	0	0	0	0	0	0
14	30	5100	328	42	48		6.4	0.87	475	0	0	0	1	1	0	0	0	0	0	0	0	0	0
15	27	9000	269	23	12	91	3.46	1.17	390	0	1	0	1	0	0	0	0	0	0	0	0	0	0
16	26.8	10600	327	19	28		3.36	0.68	290	0	0	1	1	0	0	0	0	0	0	0	1	1	0
17	30.6	8700	556	20	9	130	5.6	0.98	2640	0	0	0	1	0	1	0	0	0	0	0	0	0	0
18	27.6	5600	214	11	10	78	14.6	0.71		0	0	0	1	1	0	0	0	0	0	0	0	0	0
19	41	6600	244	18	15		1.6	0.67	171	0	0	0	1	1	0	0	0	0	0	0	0	0	0
20	32.7	6900	140	13	9		8	0.58	1119	0	0	0	1	0	0	0	0	0	0	0	1	0	0

S. No.	Adverse effects								2nd visit				Concomitant medic			
	Loss of appetite	Worsening of diarrhea	Abdominal distension	Perianal pain	Flatulence	Borborygmi	Bloating	Constipation	Slurry retention time	CDAI	HBI	SES CD	IV Steroids	Oral Prednisolone	Oral Budesonide	5-ASA
1	0	1	0	0	0	1	0	0	4	103	3	3	0	0	0	2.4
2	0	0	1	0	0	0	0	0								
3	0	0	1	0	0	1	0	0	1.5	135	1	14	0	0	0	3.6
4	0	0	0	0	0	1	1	0	3	220	4	3	0	0	6	3
5	0	0	1	0	0	0	0	0	4	130	2	7	0	0	0	0
6	0	0	0	0	0	0	0	0	3	134	1	7	0	0	9	2
7	0	0	1	0	1	0	0	0	3	130	2	2	0	0	0	0
8	0	0	1	0	0	1	0	0	2.5	73	1	6	0	0	0	3
9	0	0	0	0	0	1	0	0	3	99	1	3	0	0	9	3
10	0	0	0	0	0	0	1	0	3	106	5	3	0	0	0	3
11	0	0	0	0	1	1	0	0	1	103	3	15	0	20	0	3
12	0	0	1	0	1	1	0	0	3	103	0	6	0	20	0	3.6
13	0	0	0	0	0	0	0	0	2.5	124	3	20	0	10	0	3.6
14	0	0	0	0	0	0	0	0	2	154	1	8	0	0	0	3
15	0	0	0	0	0	1	0	0	3	177	2	11	0	1	0	3
16	0	0	0	0	0	0	0	0	3	80	1	12	0	0	9	3
17	0	0	0	0	0	1	0	0	3.5	139	2	20	0	0	0	3
18	0	0	0	0	0	0	1	0	3.5	130	3	5	0	0	0	0
19	0	0	0	0	0	1	0	0	3.5	128	1	0	0	0	0	3
20	0	0	0	0	0	0	0	0	2.5	351	5	10	0	0	0	3

S. No.	cations						Adverse effects											
	AZA	TOFA	IFX	ADA	VDZ	MTX	Fever	Pain abdomen	Nausea	Vomiting	Loss of appetite	Worsening of diarrhea	Abdominal distension	Perianal pain	Flatulence	Borborygmi	Bloating	Constipation
1	75	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
2																		
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
5	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
7	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
8	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	20	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
12	0	10	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
13	50	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
14	50	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
15	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
18	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
19	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

S. No.	3rd visit				Concomitant medications										Ad					
	Slurry retention time	CDAI	HBI	SES CD	IV Steroids	Oral Prednisolone	Oral Budesonide	5-ASA	AZA	TOFA	IFX	ADA	VDZ	MTX	Fever	Pain abdomen	Nausea	Vomiting	Loss of appetite	Worsening of diarrhea
1	3.5	80	0	3	0	0	0	2.4	75	0	0	0	0	0	0	0	0	0	0	0
2																				
3	2.5	101	1	12	0	0	0	3.6	0	0	0	0	0	0	0	0	0	0	0	0
4	3.5	212	2	3	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
5	4	134	3	8	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
6	2.5	207	1	7	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
7																				
8	3	133	4	4	0	0	0	3	0	0	0	1	0	0	0	0	0	0	0	0
9	3.2	73	1	4	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
10	3.5	112	0	3	0	0	0	3	0	0	0	0	0	0	0	1	0	0	0	0
11	1.5	149	2	15	0	0	0	3	0	10	0	0	0	0	0	0	0	0	0	0
12	3.2	103	0	6	0	0	0	3.6	0	10	0	0	0	0	0	0	0	0	0	0
13																				
14	3	255	5	8	0	0	0	3	50	0	0	0	0	0	0	0	0	0	0	0
15	3	171	1	10	0	0	0	3	50	0	0	0	0	0	0	0	0	0	0	0
16	2.5	81	1	14	0	0	6	3	0	0	0	0	0	0	0	0	0	0	0	0
17	3	124	1	15	0	0	0	3	0	10	0	0	0	0	0	0	0	0	0	0
18	3	99	3	4	0	0	0	0	50	0	0	0	0	0	0	0	1	1	0	0
19	2.5	119	1	0	0	0	0	3	100	0	0	0	0	0	0	0	0	0	0	0
20																				

S. No.	verse effects						4th visit				Concomitant medications							
	Abdominal distension	Perianal pain	Flatulence	Borborygmi	Bloating	Constipation	Slurry retention time	CDAI	HBI	SES CD	IV Steroids	Oral Prednisolone	Oral Budesonide	5-ASA	AZA	TOFA	IFX	ADA
1	0	0	0	0	0	0	4	103	3	6	0	0	0	2.4	75	0	0	0
2																		
3	0	0	0	0	0	0	2	98	2	15	0	0	0	3.6	0	0	0	0
4	0	0	0	0	0	0	3	141	4	4	0	0	0	3	0	0	0	0
5	1	0	0	1	0	0	3	115	2	9	0	0	0	0	0	0	0	1
6	0	0	1	0	1	0	3	145	3	7	0	0	0	2	0	0	0	0
7																		
8	0	0	0	0	1	0	2.5	114	1	6	0	0	0	0	0	0	0	1
9	0	0	0	1	0	0	3.5	84	1	0	0	0	0	3	0	0	0	0
10	0	0	0	0	0	0	3	55	1	3	0	0	0	3	0	0	0	0
11	0	0	0	0	0	0	1	153	3	15	0	0	0	3	0	20	0	0
12	0	0	0	0	1	0	2.8	54	1	6	0	0	0	2.4	0	10	0	0
13																		
14	0	0	0	0	0	0	2.5	236	2	10	0	0	0	3	50	0	0	0
15	0	0	0	0	0	0	3	103	1	10	0	0	0	3	50	0	0	0
16	0	0	0	1	0	0	3	78	4	8	0	0	3	3	0	0	0	0
17	0	0	0	0	0	0	3	54	0	15	0	0	0	3	0	10	0	0
18	0	0	0	0	0	0	3	149	2	4	0	0	0	0	50	0	0	0
19	0	0	0	0	1	0	3	48	0	0	0	0	0	3	100	0	0	0
20																		

S. No.			Adverse effects													
	VDZ	MTX	Fever	Pain abdomen	Nausea	Vomiting	Loss of appetite	Worsening of diarrhea	Abdominal distension	Perianal pain	Flatulence	Borborygmi	Bloating	Constipation	Fecal Cal (Post)	Follow Up SES CD
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	133	0
2																
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	527	16
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	22	
5	0	0	0	0	0	0	0	0	0	0	0	1	1	0	790	7
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	460	6
7																
8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	99	
9	0	0	0	0	0	0	0	0	0	0	0	1	0	0	127	
10	0	0	0	0	0	0	0	0	0	0	0	1	0	0	59	0
11	0	0	0	0	0	0	0	0	10	0	0	1	0	0	670	
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27	1
13																
14	0	0	0	1	0	0	0	0	0	0	0	0	1	0		
15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	169	12
16	0	0	0	1	0	0	0	0	0	0	0	1	0	0	138	7
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	370	16
18	0	0	0	0	0	0	0	0	0	0	0	1	0	0		
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
20																

Baba Farid University
of Health Sciences
Sadiq Road
Faridkot-151 203 (Pb). INDIA.
Phone : 01639-256232,256236
Tele/Fax : 01639-256235
Email – Thesisbfuhs@gmail.com

(82)

No. BFUHS/2K23p-TH/72 Date 01/01/24

The Principal
Dayanand Medical College & Hospital
Ludhiana

Subject:: Approval of Plan of Thesis.

This is to inform you that the Faculty of Medical Sciences in its meeting held on 25.09.2023 has approved the plan of thesis of **Shivam Bansal,** a student of your college for **DM Medical Gastroenterology** along with the names of Supervisor/Co-Supervisor as under:-

Topic Plan of Thesis
Fecal microbiota transplantation for induction of remission in mild to moderate active crohn's disease .

Supervisor
Dr. Ajit Sood
Prof. & Head
Dept. of Gastroenterology

Co-Supervisor:-
Dr. Ramit Mahajan
Assoc. Professor
Dept. of Gastroenterology

The student may submit the thesis in triplicate alongwith the plan in triplicate also, to the Controller of Examinations, BFUHS, Faridkot, at least six months before the commencement of theory examination, if he/she is otherwise eligible to take the examination. He /She may be directed to attach a photocopy of this letter with the forwarding letter while submitting thesis in the University along with the letter of approval of University for change of supervisor/Co-Supervisor, change of topic, if any.

Controller of Examinations

Note :

1. *If any discrepancy found regarding topic of thesis or Supervisor/Co-Supervisor, inform undersigned within one month after issue of this letter.*

FECAL MICROBIOTA TRANSPLANTATION FOR INDUCTION OF REMISSION IN MILD TO MODERATE ACTIVE CROHN'S DISEASE

THESIS PROPOSAL

FOR APPROVAL OF SUBJECT OF THESIS TO BE SUBMITTED

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

DM

IN

GASTROENTEROLOGY

OF THE

BABA FARID UNIVERSITY OF HEALTH SCIENCES
FARIDKOT

NOVEMBER, 2022

DR. SHIVAM BANSAL

DEPARTMENT OF GASTROENTEROLOGY
DAYANAND MEDICAL COLLEGE &HOSPITAL
LUDHIANA

ABSTRACT OF PLAN OF THESIS

Title	FECAL MICROBIOTA TRANSPLANTATION FOR INDUCTION OF REMISSION IN MILD TO MODERATE ACTIVE CROHN'S DISEASE
For the degree of	DM Gastroenterology
Name of the candidate	Dr. Shivam Bansal
Supervisor	Dr. Ajit Sood, Professor and Head, Department of Gastroenterology, DMC Ludhiana
Co-supervisor	Dr. Ramit Mahajan , Associate Professor, Department of Gastroenterology, DMC Ludhiana
Institution	Dayanand Medical College & Hospital, Ludhiana
University	Baba Farid University of Health Sciences, Faridkot

Introduction: The current consensus in IBD includes an inter-play of aberrations of innate and adaptive immune responses, host genetics, changes in luminal microbiota and environmental influences. Despite the best evidence-based practice, only about half of all patients with CD achieve sustained remission with medical therapies. Microbiota targeted therapies like fecal microbiota transplantation (FMT) present an alternative therapeutic approach that decrease the antigenic drive that fuels the downstream aberrant immune responses

Aim of Study: The primary objective of the current study will be to determine the efficacy of FMT compared to placebo in inducing clinical remission in patients with mild to moderate active CD. The secondary objectives will include clinical, radiological and endoscopic response at the end of the study period.

Materials & methods:A randomised, double-blind, placebo-controlled trial of FMT in patients with mild to moderate active CD will be conducted at Dmch.Ludhiana. Patients will be selected after fulfilment of inclusion and exclusion criteria. Stool from healthy donors will be infused via a colonoscope in terminal ileum and cecum within 6 hours of collection of stool and 1 hour of preparation of the slurry. Blood samples will be collected at baseline, week 8 and week 16 for complete blood counts C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and renal and liver function tests. Fecal calprotectin will be performed at baseline, week 8 and week 16. Fecal samples will be collected longitudinally from the patients at baseline and week 16 for microbiota analysis.

Clinical Significance: Despite having the highest number of IBD patients in the world, there is limited affordability and access to biologics due to their probative cost and lack of insurance cover. Additionally, an increased risk of infections, especially tuberculosis, restricts the use of biologics. Alternative therapies are hence actively sought by the IBD physicians in India. FMT appears an attractive adjunct to the standard of care pharmacotherapy as it targets the key drivers of inflammation, the gut microbiota.

Keywords: IBD , CD, Fecal Microbiota transplant, Crohn disease activity index, SES-CD score, MARIA score, Fecal microbiota analysis

(Signature) Candidate	(Signature) Co-Supervisor	(Signature) Supervisor	(Signature) Principal

CERTIFICATE OF FACILITIES AVAILABLE

This is to certify that the facilities for work on the subject of thesis titled **"FECAL MICROBIOTA TRANSPLANTATION FOR INDUCTION OF REMISSION IN MILD TO MODERATE ACTIVE CROHN'S DISEASE"** exist in the Department of Gastroenterology, DMC&H, Ludhiana and will be provided to the candidate. We will see that the data being included in the thesis is genuine and is collected by the candidate himself under our supervision and guidance.

Supervisor

Dr. Ajit Sood
Professor & Head
Department of Gastroenterology
DMCH, Ludhiana

Co-Supervisor

Dr. Ramit Mahajan
Associate Professor
Department of Gastroenterology
DMCH, Ludhiana

Head of the Department

Dr. Ajit Sood
Professor & Head
Department of Gastroenterology
DMCH, Ludhiana

Place : _____

Date : _____

APPROVAL PROFORMA
BY RESEARCH & ETHICAL COMMITTEE
DAYANAND MEDICAL COLLEGE & HOSPITAL, LUDHIANA

Name of candidate	Dr. Shivam Bansal
Department	DM Gastroenterology
Topic of Thesis	FECAL MICROBIOTA TRANSPLANTATION FOR INDUCTION OF REMISSION IN MILD TO MODERATE ACTIVE CROHN'S DISEASE
Likely date of appearing in PG examination	June 2025
Date of enrollment	27th April, 2022
Name of Head of Department	Dr. Ajit Sood
1) Supervisor **2) Co-Supervisor**	Dr. Ajit Sood Dr. Ramit Mahajan
Signature of Members of Research Committee with Stamp	**Signature of Members of Ethics Committee with Stamp**
Dr. Harpreet Kaur (Professor & Head, Pathology)	Mr. R K Sharma (Legal Expert)
Dr. Jaspal Singh (Professor & Head, Surgery)	Dr. Ramit Mahajan (Associate Professor, Gastroenterology)
Dr. Dinesh Gupta (Professor & Head, Medicine)	Dr. Anu Sharma (Professor, Anatomy)
Dr. Sandeep Kaushal (Dean Academics & Professor & Head, Pharmacology)	Dr. Sandeep Kaushal (Dean Academics & Professor & Head, Pharmacology)
Dr. Gurpreet S Wander (Vice Principal)	Dr. Gurpreet S Wander (Vice Principal & Professor & Head, Cardiology)
	Dr. Gagandeep Singh (Secretary, Ethics Committee Professor & Head, Neurology)

Approved: Yes / ~~No~~

Dr. Sandeep Puri
Chairperson
Research Committee
Chairperson
Research Committee

Approved: Yes / No

Prof. Arvind Malhotra
Chairperson
Ethics Committee
Chairperson
Ethics Committee

BABA FARID UNIVERSITY OF HEALTH SCIENCES, FARIDKOT

PROFORMA FOR REGISTRATION OF SUBJECT FOR DISSERTATION

1	NAME OF THE CANDIDATE AND ADDRESS	DR. SHIVAM BANSAL ADDRESS- #9, KALINDI COLONY, YAMUNANAGAR, HARYANA 135001
2	NAME OF THE INSTITUTE	DAYANAND MEDICAL COLLEGE AND HOSPITAL
3	COURSE OF STUDY AND SUBJECT	D.M. GASTROENTEROLOGY
4	DATE OF ADMISSION TO COURSE	27/04/22
5	TITLE OF THE TOPIC	FECAL MICROBIOTA TRANSPLANTATION FOR INDUCTION OF REMISSION IN MILD TO MODERATE ACTIVE CROHN'S DISEASE

6.0 BRIEF RESUME OF THE INTENDED WORK

6.1 NEED FOR STUDY

Inflammatory bowel disease (IBD) includes ulcerative colitis (UC) and CD. Both UC and CD are chronic, relapsing, and remitting inflammatory diseases associated with significant morbidity and mortality.[1] While the exact etiology of IBD is not clear, the understanding of the pathogenesis of these diseases has evolved and the current consensus includes an inter-play of aberrations of innate and adaptive immune responses, host genetics, changes in luminal microbiota and environmental influences.[2]

The therapeutic approaches for treatment of IBD target one of these potential offenders. The state of art medical therapy aims to correct the aberrant immune responses by utilizing a variety of drugs including anti-inflammatory agents (corticosteroids, 5-aminosalicylates), immune-suppressants (thiopurines, methotrexate, etc.), biologics (anti-tumor necrosis factor agents, anti integrins, anti interleukin 12/23, anti-IL-23 agents, etc.) and small molecules (JAKinibs S1P modulators, etc.).[3] Despite the best evidence-based practice, only about half of all patients with CD achieve sustained remission with these therapies.[4]

Alterations to the gut microbiota have been closely linked to intestinal inflammation in IBD and this has led to the hypothesis that IBD develops due to quantitative and qualitative microbial imbalance in the gut (dysbiosis). The gut microbiota is theorized to be a key antigenic driver of the aberrant inflammatory responses in IBD.[2] While medical therapy with drugs suppresses the aberrant immune response in patients with IBD, microbiota targeted therapies like fecal microbiota transplantation (FMT) present an alternative therapeutic approach that decrease the antigenic drive that fuels the downstream aberrant immune responses.[5]

As a result, there is a growing interest and intensified research in recent years, primarily focusing on the bacterial component of the microbiome. The FMT employs administration of *healthy donor whole stool* (that consists of microbial communities and their functional ecologies) into the gastrointestinal tract of an individual with the aim of correcting dysbiosis.[6] However, the precise mechanism by which FMT works in IBD is still not clear. Dysbiosis in IBD is characterized by reduction in *Bacteroidetes*, reduced diversity within *Firmicutes* and an increased proportion of *Proteobacteria*.[7] A microbial signature for CD characterized by decreased representation of *Faecalibacterium*, *Ruminococcaceae*, and *Bacteroides*; and increased presence of *Gammaproteobacteria* and *Fusobacteriaceae* has been described. FMT is expected to correct this dysbiosis and thereby reduce gut inflammation in UC.[8] Further, the microorganisms within the intestinal tract not only synergistically cooperate in nutrient digestion and metabolism but also intensely compete with each other for nutrients and space. The exclusion of competitive niche by FMT-induced restoration of microbial communities (and thereby preventing opportunistic pathogens to persist and proliferate) is another plausible mechanisms for its therapeutic effect. Fmt also targets the immune responses in patients with IBD. Animal models have shown reduced colonic inflammation following FMT due to reestablishment of colonic CD4+ and Treg cells, increase in IL-10 production, reduced ability of antigen presenting cells to present bacterial antigens to the colonic T cells and restoration of intestinal memory/effector T cell populations. While FMT has been found efficacious and safe in randomized clinical trials in patients with active UC, data are limited for patients with CD.[9]

6.2 REVIEW OF LITERATURE-

Modifying the composition of the intestinal microbiota in order to overcome gut dysbiosis have become a major interest in recent years. Convincing data and studies are available regarding the use and efficacy of FMT in recurrent Clostridium Difficile infection and ulcerative colitis but there is limited evidence for use of FMT in Crohn's disease.

A randomized, single-blind, sham-controlled pilot trial was done by Sokol et al to demonstrate role of FMT in adults with colonic or ileocolonic CD. Patients enrolled while in flare received oral corticosteroid. Once in clinical remission, patients were randomized to receive either FMT or sham transplantation during a colonoscopy. Corticosteroids were tapered and a second colonoscopy was performed at week 6. The primary endpoint was the implantation of the donor microbiota at week 6 (Sorensen index > 0.6).Total patients enrolled were 17. The steroid-free clinical remission rate at 10 and 24 weeks was 44.4% (4/9) and 33.3% (3/9) in the sham transplantation group and 87.5% (7/8) and 50.0% in the FMT group. Crohn's Disease Endoscopic Index of Severity decreased 6 weeks after FMT ($p = 0.03$) but not after sham transplantation ($p = 0.8$). Conversely, the CRP level increased 6 weeks after sham transplantation ($p = 0.008$) but not after FMT ($p = 0.5$).[1]

In a randomized controlled trial conducted by Yang et al evaluating the comparative efficacy of FMT delivered by gastroscopy or colonoscopy. Of the 27 patients included in the study, clinical remission was achieved in 18 (66.7%); no significant difference wa seen between the two methods. 76.9% of gastroscopy group patients and 64.3% of colonoscopy group patients experienced mild adverse events during or shortly after treatment. Microbiota diversity analyses showed that, in comparison with the donors, patients had lower operational taxonomic units (OTU; 117 vs. 258, $p < 0.05$) and Shannon diversity index (2.05 vs 3.46, $p < 0.05$). The CD

patients showed a significant increase in OTU and Shannon diversity index 2 weeks after FMT. In comparison with the donors, CD patients had lower levels of *Bacteroides*, *Eubacterium*, *faecalibacterium*, and *Roseburia*, and higher levels of *Clostridium*, *Cronobacter*, *Fusobacterium*, and *StreptococcuS*.[10]

A study conducted by Xiang et al included 173 patients with median follow up of 43 months. Seven therapeutic targets included abdominal pain, diarrhoea, hematochezia, fever, steroid-dependence, enterocutaneous fistula and active perianal fistula. Each target was recorded as 1 (yes) or 0 (no) during the long-term follow-up for each patient. The median score of the total targets was 2 (range, 1–4) before FMT, and it decreased significantly at 1, 3, 6, 12, 24 and 36 months after FMT ($P < 0.001$ respectively). At 1 month after FMT, 72.7% (101/139), 61.6% (90/146), 76% (19/25) and 70.6% (12/ 17) of patients achieved improvement in abdominal pain, diarrhoea, hematochezia and fever respectively. Furthermore, 50% (10/20) of steroid-dependent patients achieved steroid-free remission after FMT.[11]

In a systematic review by Fehily et al, The majority of trials evaluated FMT for induction of remission, with follow-up duration varying from 4 to 52 weeks. One RCT in 21 patients, of single-dose FMT *versus* placebo, following steroid-induced remission, showed a higher rate of steroid-free clinical remission in the FMT group compared to the control group: 87.5% *vs* 44.4% at week 10 ($P = 0.23$). Another RCT, two-dose FMT in 31 patients, showed an overall clinical remission rate of 36% at week 8, however, with no difference in clinical or endoscopic endpoints between FMT administered by gastroscopy and colonoscopy. Considering all studies, the clinical response rates in early follow up were higher following multiple FMT than with single FMT. FMT dose did not appear to influence clinical outcomes, nor did whether FMT was fresh or frozen. FMT

delivered via upper gastrointestinal route demonstrated higher early efficacy rates of 75 to 100% compared with lower delivery route rates of 30% to 58%, but on follow up beyond 8 weeks, this difference was not maintained. Whether pre-FMT antibiotic administration was beneficial was not able to be determined due to the limited number of patients receiving antibiotics and varying antibiotic regimens. No serious adverse events were reported.[4]

However the FMT protocols differed substantially reflecting the absence of universal guidelines for FMT in terms of preparation, route of delivery, dosing and frequency. Most of the case series/cohort studies have performed a single session of FMT. But, because of complex and persistent pathologic mechanisms in CD, the therapeutic microbial manipulation with a single session of FMT is unlikely to have sustained benefits.[12]

In one of the study median time for maintaining clinical response with FMT in Crohn's disease patients was about 4 months. Higher response rates were described in studies that administered multiple FMTs.[13] Subgroup analyses from systematic reviews evaluating FMT in CDI have demonstrated increased efficacy associated with multiple FMTs and that the rate of clinical remission with fresh stool FMT was higher than with frozen stool FMT (73% vs 43%; $p < 0.05$).[14] Most of the adverse events reported with FMT have been minor and self-resolving and no major FMT-related adverse events have been reported so far.[3]

6.3 AIMS AND OBJECTIVES-

In this study, we will be targeting both immune and microbiota components of the disease pathogenesis, i.e. FMT will be used as an add-on therapy to the standard of care.

Primary outcome

1. *Clinical remission (defined as CDAI <150) at week 20*
2. *Endoscopic remission (SES CD< 3) at week 20*

Secondary outcomes

1. Clinical response (defined as decline in CDAI by > 100 points) at week 20
2. Endoscopic response (defined as >50% decline in SES CD) at week 20

7.0 MATERIAL AND METHODS:

7.1 SOURCE OF DATA:

Study Design

A randomised, double-blind, placebo-controlled trial of FMT in patients with mild to moderate active CD will be conducted at Dayanand Medical College and Hospital, Ludhiana, India.

Study Place- Dayanand Medical College and Hospital, Ludhiana, India.

7.2 METHOD OF COLLECTION OF DATA

Disease definition, classification and assessment of disease activity

Adult patients with established diagnosis of CD (based on the European Crohn's and Colitis Organisation [ECCO] and the European Society of Gastrointestinal and Abdominal Radiology [ESGAR] guidelines) will be included. Montreal classification will be used for classifying CD. Clinical disease activity will be assessed by Crohn's disease activity index (CDAI).

Clinical remission will be defined as CDAI score < 150 points, while mild to moderate disease will be defined as CDAI 150-449 points. Clinical response will be defined as a decline in CDAI by >100 points. Endoscopic disease activity will be assessed by simple endoscopic score for CD (SES-CD). SES CD ≥6 will indicates active disease. Endoscopic response will be defined by a 50% reduction in the SES-CD score from baseline. (Table 1)

Montreal Classification for adult CD		
Parameters		**Description**
Age at diagnosis	A1	Below 16 years
	A2	Between 17 and 40 years
	A3	Above 40 years
Location	L1	Ileal

	L2	Colonic
	L3	Ileocolonic
	L4	Isolated upper disease*
Behavior	B1	Non-stricturing, non-penetrating
	B2	Stricturing
	B3	Penetrating
	P	Perianal disease modifier†
*L4 is a modifier that can be added to L1-3 when concomitant upper gastrointestinal disease is present. † "P" is added to B1-B3 when concomitant perianal disease is present.		

Crohn's Disease Activity Index (CDAI)	
Clinical or laboratory variable	Weighting factor
Number of liquid stools or soft stools each day for 7 days	2
Abdominal pain (graded from 0 to 3 based on severity) each day for 7 days	5
General well being, subjectively assessed from 0 (well) to 4 (terrible) each day for 7 days	7
Complications*	20
Use of diphenoxylate or opiates for diarrhea	30
An abdominal mass (0 for none, 2 for questionable, 5 for definite)	10
Absolute deviation of haematocrit from 47% in men and 42% in women	6
Percentage deviation from standard weight	1
*One point is added for each set of complications: arthralgia or frank arthritis, inflammation of the iris, sclera or uveitis, erythema nodosum, pyoderma gangrenosum, aphthous ulcers, anal fissures, fistulas, abscesses, other fistulas, and fever (>100 F) during the previous week	

Simple endoscopic score for Crohn's disease				
Variable	Score			
	0	1	2	3
Size of ulcers	None	Aphthous ulcers (diameter 0.1-0.5 cm)	Large ulcers (diameter 0.5-2 cm)	Very large ulcers (diameter > 2 cm)
Ulcerated surface	None	<10%	10-30%	>30%
Affected surface	Unaffected segment	<50%	50-75%	>75%
Presence of	None	Single, can	Multiple, can	Cannot be

narrowings		be passed	be passed	passed
Total SES CD : sum pf the values of the four variables for the 5 bowel segments (ileum, right colon, transverse colon, left colon and rectum) SES CD ≥6 indicates active disease. Endoscopic response is defined by a 50% reduction in the SES-CD score from baseline.				

INCLUSION CRITERIA-

1. Patients with mild to moderate active CD, defined as CDAI 150-449 and SES CD ≥6 (endoscopic assessment performed within 12 weeks of screening)
2. Ileo-colonic or colonic disease location
3. Inflammatory (non penetrating non structuring disease behavior)
4. Diagnosis (endoscopic or radiographic and histological) of IBD at least 6 months prior to enrolment into the study
5. Age ≥ 18 years
6. Subjects who are willing and able to comply with treatment plan, laboratory tests.
7. Subjects who are willing to provide a written informed consent.

EXCLUSION CRITERIA-

1. Patients with severe CD (CDAI>450)
2. Isolated ileal disease
3. Stricturing or fistulizing disease behavior
4. Perianal fistulizing disease
5. SES CD <6
6. Subjects without previous treatment for IBD (i.e. treatment-naïve)
7. Active or latent or inadequately treated infections (includingClostridiodes difficile, Cytomegalovirus, Mycobacterium tuberculosis, etc.)
8. Subjects displaying clinical signs of fulminant colitis or toxic megacolon

9. Subjects who received antibiotics or probiotics within 4 weeks of screening
1. Subjects on concomitant therapy with an anti-tumour necrosis factor (TNF) agent (infliximab, adalimumab, or golimumab) or anti integrins or ustekinumab
10. Subjects infected with human immunodeficiency virus (HIV) or hepatitis B or C viruses
11. Subjects who have been vaccinated with live or attenuated vaccine within 6 weeks of baseline or scheduled to receive these vaccines during study period or within 6 weeks after last FMT session
12. Subjects with malignancies or a history of malignancies
13. Subjects with current or recent history of severe, progressive, or uncontrolled renal, hepatic, haematological, gastrointestinal, metabolic, endocrine, pulmonary, cardiac, neurological disease.
14. Pregnant females
15. Age <18 years

Permitted therapies

1. Oral mesalazine (stable dose for ≥4 weeks)
2. Thiopurines or methotrexate (stable dose for ≥4 weeks)
3. Oral prednisolone or equivalent (dose ≤20 mg daily).
4. Patients were required to continue stable doses of all CD therapies during the trial period except for prednisolone, which had a mandated tapering of 5 mg weekl to ensure corticosteroid-free status by week 4.

Selection and Screening of Stool Donor

A preliminary interview of the prospective donor will be performed by a structured questionnaire that will investigate several risk factors to minimize the risk of transferring infections or adverse gut microbiota profile. In the medical interview screening, potential donors will be inquired

about the use of drugs that can alter gut microbiota, known history or risk behaviors for infectious disease, and for disorders potentially associated with the disruption of gut microbiota.

Donor stool will be tested for enteric pathogens including parasites (Cryptosporidium, Entamoeba histolytica, Giardia, Cyclospora), bacteria (Salmonella, Vibrio, Plesiomonas, Shigella, Escherichia coli, Campylobacter, Yersinia, and Clostridiodes difficile) and viruses (Adenovirus, Astrovirus, Norovirus, Rotavirus and Sapovirus). Additionally stool cultures for Salmonella, Shigella, Escherichia coli, Campylobacter, Yersinia will also be done. The donor will also be tested for hepatitis A, hepatitis B, hepatitis C, hepatitis E, human immunodeficiency virus, cytomegalovirus (IgM) and syphilis. A short questionnaire will be administered on the same day of the donation to check for any recent-onset harmful events.

Minimum criteria for donor selection

- Age > 20 years and < 50 years
- 27 kg/m^2 > body mass index > 17 kg/m^2 (body mass index is defined as the body mass divided by the square of the body height)
- No co-morbid illness associated with gut microbial dysbiosis
- Vegetarian
- Regular bowel movements

Randomization

Patients will be randomized in a 1:1 ratio to receive either FMT or placebo, using a pre-established computer-generated randomisation list. The individual who will perform the randomisation will not be otherwise involved in the trial.

Preparation and administration of fecal slurry

A freshly collected sample was will be inspected visually and formed stools (Bristol stool scale, 3 and 4) with no obvious blood or mucous will be accepted. This sample will be diluted with preservative free normal saline and homogenized using a blender (Stomacher® 400 circulator; Seward Ltd, Worthing, UK) till it reaches a liquid consistency. Universal precautions will be observed and separate disposable bags will be used for each patient. The stool slurry will be filled in four 50 mL syringes after filtering out the particulate matter and infused via a colonoscope in terminal ileum and cecum within 6 hours of collection of stool and 1 hour of preparation of the slurry. The recipients will be encouraged to retain stool slurry for as long as possible and observed for 6 hours to assess stool retention time and immediate post-procedure adverse events.

Investigations

Blood samples will be collected at baseline, week 8 and week 16 for complete blood counts (including hemoglobin, leucocyte count and platelets), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and renal and liver function tests. Fecal calprotectin will be performed at baseline, week 8 and week 16.

Diet

Diet is one of the most important determinants of gut microbial composition. (44-46) It has been proposed that the habitual diet consumed over the long term decides the fundamental microbial health. (12) Complex interactions between dietary nutrients and microbiota shape the immune responses that trigger/sustain the disease. Maintaining a healthy diet is hence important for both the donor and the recipient of FMT. The recipients of FMT will be on a controlled diet during the trial period.

DURATION OF THE STUDY- One year, 1st January,2023 to 31st December, 2023

Sample size

We expected a true mean difference of 30% in the treatment and the control group. Using α error rate of 5% and 80% power of the study, δ (clinically meaningful difference) of 20 (response rates to FMT vary between 50-80%) and a 30% dropout rate; an estimated 36 patients (18 in each group) will be required for inclusion.

OUTCOME:

Primary outcome

3. *Clinical remission (defined as CDAI <150) at week 20*
4. *Endoscopic remission (SES CD< 3) at week 20*

Secondary outcomes

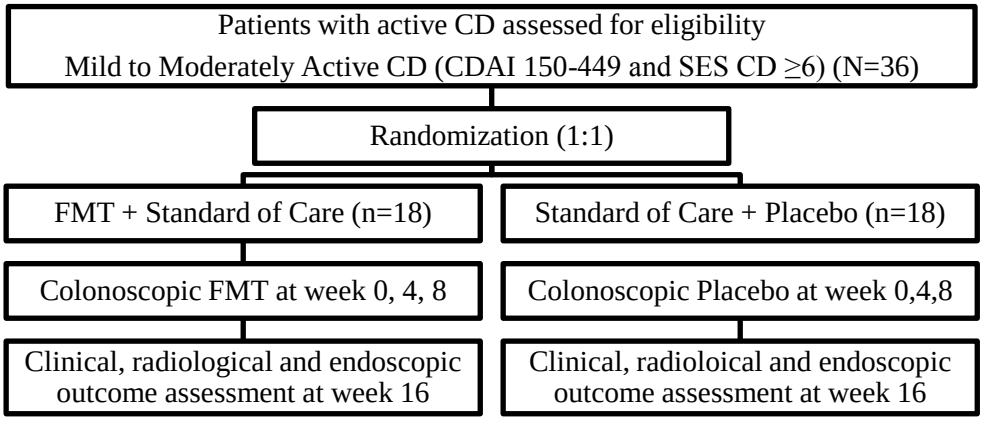
3. Clinical response (defined as decline in CDAI by > 100 points) at week 20
4. Endoscopic response (defined as >50% decline in SES CD) at week 20

STATISTICAL ANALYSIS

The statistical analysis will follow the intention-to-treat approach and results will be reported according to the CONSORT statement recommendations. Patients' characteristics will be described for the total cohort and for each group of randomization. Qualitative data will be expressed as number (%) and quantitative data will be expressed as medians and interquartile range (IQR). The primary and secondary outcomes will be compared between the two groups with a Fisher's exact test. Quantitative variables will be compared using a non-parametric

Wilcoxon rank-sum tests. The similarity of the fecal microbiota samples will be assessed using the Sorensen similarity index (Sorensen similarity index = [1 – Bray Curtis dissimilarity index]). The non-parametric Wilcoxon rank-sum test will be used to compare the similarity indices between groups.

Flow of the Study


Patients with active CD assessed for eligibility
Mild to Moderately Active CD (CDAI 150-449 and SES CD ≥6) (N=36)
Randomization (1:1)
FMT + Standard of Care (n=18)
Standard of Care + Placebo (n=18)
Colonoscopic FMT at week 0, 4, 8
Colonoscopic Placebo at week 0,4,8
Clinical, radiological and endoscopic outcome assessment at week 16
Clinical, radioloical and endoscopic outcome assessment at week 16

	Pre screening period	Screening Visit	Visit 1	Visit 2	Visit 3	Follow Up Visit 4
	Between -12 weeks of the baseline (week 0) visit	Between -4 weeks and baseline (week 0) visit	Week 0	Week 4	Week 8	Week 16
Clinical history		✓	✓	✓	✓	✓
Screening colonoscopy	✓					
MR Enterography		✓				✓
Intervention (FMT/Placebo)			✓	✓	✓	
Complete blood count		✓			✓	✓
Renal function tests		✓			✓	✓
Liver function		✓			✓	✓

tests						
C reactive protein		✓			✓	✓
Erythrocyte sedimentation rate		✓			✓	✓
Fecal Calprotectin		✓			✓	✓
Fecal sample collection for microbiota analysis		✓			✓	✓
Colonoscopy for outcome assessment						✓

7.3 DOES THE STUDY REQUIRE ANY INVESTIGATIONS OR INTERVENTIONS TO BE CONDUCTED ON PATIENTS OR OTHER HUMANS OR ANIMALS? IF SO, PLEASE DESCRIBE BRIEFLY.

In this study, Fecal microbiota transplantation will be done in patients fulfilling inclusion criteria. Revelant blood and stool investigations will also be done.

7.4 HAS ETHICAL CLEARANCE BEEN OBTAINED FROM YOUR INSTITUTION IN CASE OF 7.3?

Yes, Attached herewith.

8. LIST OF REFERENCES

1. Sokol H, Landman C, Seksik P, Berard L, Montil M, Nion-Larmurier I,et al. Fecal microbiota transplantation to maintain remission in Crohn's disease: a pilot randomized controlled study. Microbiome. 2020;8:1-4.

2. Cheng F, Huang Z, Wei W, Li Z. Fecal microbiota transplantation for Crohn's disease: a systematic review and meta-analysis. Tech Coloproctology. 2021;25:495–504.

3. Wang H, Cui B, Li Q, Ding X, Li P, Zhang T, et al. The Safety of Fecal Microbiota Transplantation for Crohn's Disease: Findings from A Long-Term Study. Adv Ther. 2018;35:1935–44.

4. Fehily SR, Basnayake C, Wright EK, Kamm MA. Fecal microbiota transplantation therapy in Crohn's disease: Systematic review. J Gastroenterol Hepatol. 2021;36:2672–86.

5. Gutin L, Piceno Y, Fadrosh D, Lynch K, Zydek M, Kassam Z, et al. Fecal microbiota transplant for Crohn disease: A study evaluating safety, efficacy, and microbiome profile. United Eur Gastroenterol J. 2019;7:807–14.

6. Fang H, Fu L, Wang J. Protocol for Fecal Microbiota Transplantation in Inflammatory Bowel Disease: A Systematic Review and Meta-Analysis. BioMed Res Int. 2018 13;2018:1–11.

7. Collins M, DeWitt M. Fecal microbiota transplantation in the treatment of Crohn disease. J Am Acad Physician Assist. 2020;33:34–7.

8. Popov J, Hartung E, Hill L, Chauhan U, Pai N. Pediatric patient and parent perceptions of fecal microbiota transplantation for the treatment of ulcerative colitis. Journal of Pediatric Gastroenterology and Nutrition. 2021;73:684-8.

9. Li Q, Ding X, Liu Y, Marcella C, Dai M, Zhang T, et al. Fecal Microbiota Transplantation is a Promising Switch Therapy for Patients with Prior Failure of Infliximab in Crohn's Disease. Front Pharmacol. 2021;12:658087.

10. Yang Z, Bu C, Yuan W, Shen Z, Quan Y, Wu S, et al. Fecal Microbiota Transplant via Endoscopic Delivering Through Small

Intestine and Colon: No Difference for Crohn's Disease. Dig Dis Sci. 2020;65:150–7.

11. Xiang L, Ding X, Li Q, Wu X, Dai M, Long C, et al. Efficacy of faecal microbiota transplantation in Crohn's disease: a new target treatment? Microb Biotechnol. 2020 ;13:760–9.

12. He Z, Li P, Zhu J, Cui B, Xu L, Xiang J, et al. Multiple fresh fecal microbiota transplants induces and maintains clinical remission in Crohn's disease complicated with inflammatory mass. Sci Rep. 2017;7:4753.

13. Suskind DL, Brittnacher MJ, Wahbeh G, Shaffer ML, Hayden HS, Qin X, et al. Fecal Microbial Transplant Effect on Clinical Outcomes and Fecal Microbiome in Active Crohn's Disease: Inflamm Bowel Dis. 2015;21:556–63.

14. Li P, Zhang T, Xiao Y, Tian L, Cui B, Ji G, et al. Timing for the second fecal microbiota transplantation to maintain the long-term benefit from the first treatment for Crohn's disease. Appl Microbiol Biotechnol. 2019;103:349–60.

15. D'Amico F, Chateau T, Laurent V, Danese S, Peyrin-Biroulet L. Which MRI Score and Technique Should Be Used for Assessing Crohn's Disease Activity? J Clin Med. 2020;9:1691.

PROFORMA

Patient ID Date

MRD Number Donor ID

Name Age Sex

Address

Contact number

Month and Year of diagnosis of CD

Duration of disease

Family history of IBD: Yes/No (If Yes, specify)

Appendicectomy: Yes/No

Addictions

Smoking No/Occassional/Regular/Heavy

Alcohol No/Occassional/Moderate/Regular

Opium No/Occassional/Regular

IVDU Yes/No

Comorbidity

HTN/T2DM/BA/CAD/COAD/Thyroid disorder/CVA/Malignancy/Psychiatric illness/ Seizures/ Others

BMI

Slurry retention time

Baseline Assessment

Crohn's Disease	
Classification	A L B P
CDAI	
HBI	
SES CD	

Signature of Candidate **Signature of Supervisor**

Previous treatments:

	Dose
IV steroids	
Prednisolone (oral)	
Budesonide (oral)	
5-ASA	
AZA/6MP	
Tofacitinib	
Infliximab	
Adalimumab	
Vedolizumab	
Methotrexate	
Antibiotics	
Probiotics	
Others	

Details of visits:

	Visit 1	Visit 2	Visit 3	Visit 4
DATE				
DISEASE ACTIVITY				
CDAI				
HBI				
SES CD				
INVESTIGATIONS				
Hb/TLC/Platelets				
MCV				
TLC				
Platelets				
AST		X	X	X

Signature of Candidate **Signature of Supervisor**

ALT		X	X	X
ALP		X	X	X
ESR		X	X	X
Albumin		X	X	X
CRP-Quantitative		X	X	X
Creatinine		X	X	X
RBS		X	X	X
HBsAg/Anti HCV/Anti HIV		X	X	X
Fecal calprotectin		X	X	
ADVERSE EFFECTS				
Fever				
Pain abdomen				
Nausea				
Vomiting				
Loss of appetite				
Worsening of diarrhea				
Abdominal distension				
Perianal pain				
Flatulence				
Borborygmi				
Bloating				
Rectal pain				
Constipation				
UTI				
URI				
LRTI				

Signature of Candidate **Signature of Supervisor**

CONCOMITANT MEDICATIONS				
Drug 1/Dose				
Drug 2/Dose				
Drug 3/Dose				
Drug 4/Dose				
Drug 5/Dose				

Signature of Candidate **Signature of Supervisor**

INFORMED PATIENT CONSENT

Date: _____

I……..D/o, W/o, S/o……. ……am willing to participate in the study **"FECAL MICROBIOTA TRANSPLANTATION FOR INDUCTION OF REMISSION IN MILD TO MODERATE ACTIVE CROHN'S DISEASE"** The procedure with its complications has been explained to me in local vernacular language. I have fully understood that no special tests and extra expenditure in any form, merely for the purpose of thesis is being carried out. I am free to opt out the study any time without assigning any reason.

……. ……

Signature of the Witness Signature of the patient

Name:

Signature of Candidate **Signature of Supervisor**

मरीज अनुमति फार्म

तिथि _____

मैं _____ पत्नी/पुत्र/पुत्री _____

निवासी _____

इस शोधकार्य **"FECAL MICROBIOTA TRANSPLANTATION FOR INDUCTION OF REMISSION IN MILD TO MODERATE ACTIVE CROHN'S DISEASE"** में भाग लेने के लिए सहमत हूँ। मैं इस शोधकार्य में भाग लेने के लिए अपनी स्वेच्छा से तैयार हूँ। मैं पुष्टि करता/करती हूँ की यह स्वीकृति देने के लिए मुझे कोई आर्थिक प्रलोभन नहीं दिया गया और न ही दबाव डाला गया है। मुझे इस शोधकार्य का उद्देश्य स्पष्ट कर दिया गया है और मुझे यह विश्वास है की यह विज्ञान और मानवता के लाभ के लिए है। मुझे इस शोधकार्य में होने वाले जोखिम के बारे में समझा दिया गया है और मेरी समझ में यह जोखिम बहुत मामुली है।

मैं इस शोधकार्य में भाग लेने के सम्बन्ध में किसी भी समय अपनी अस्वीकृति व्यक्त कर सकता/सकती हूँ और मेरे अस्वीकृति व्यक्त करने का मेरे इलाज पर कोई प्रभाव नहीं पड़ेगा।

_____ _____

गवाह के हस्ताक्षर रोगी के हस्ताक्षर/अंगूठे का निशान

नाम: नाम:

_____

अन्वेषक केहस्ताक्षर

_____

विद्यार्थी के हस्ताक्षर

ਮਰੀਜ਼ ਰਜ਼ਾਮੰਦੀ ਫਾਰਮ

ਮਿਤੀ_____

ਮੈ_____ਪਤਨੀ/ਪੁੱਤਰ/ਪੁੱਤਰੀ_____

ਵਾਸੀ _____

“FECAL MICROBIOTA TRANSPLANTATION FOR INDUCTION OF REMISSION IN MILD TO MODERATE ACTIVE CROHN’S DISEASE” ਤੇ ਚਲ ਰਹੀ ਖੋਜ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਰਜ਼ਾਮੰਦ ਹਾਂ। ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਲਈ ਮੈ ਆਪਣੀ ਮਰਜ਼ੀ ਨਾਲ ਤਿਆਰ ਹਾਂ। ਮੈ ਇਹ ਦਸਣਾ ਚਾਹੁੰਦਾ ਹਾਂ/ ਚਾਹੁੰਦੀ ਹਾਂ ਕੇ ਇਹ ਰਜ਼ਾਮੰਦੀ ਦੇਣ ਵਾਸਤੇ ਮੈਨੂ ਕਿਸੇ ਵੀ ਤਰਾਂ ਦਾ ਲੋਭ ਲਾਲਚ ਨਹੀ ਦਿੱਤਾ ਗਿਆ ਅਤੇ ਨਾਹੀ ਕੋਈ ਦਬਾ ਪਾਇਆ ਗਿਆ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਦਾ ਮਨੋਰਥ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂਇਹ ਭਰੋਸਾ ਦਵਾਇਆ ਗਿਆ ਹੈ ਕੇ ਇਹ ਖੋਜ ਵਿਗਿਆਨ ਅਤੇ ਮਾਨਵਤਾ ਦੀ ਭਲਾਈ ਲਈ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਵਿਚ
ਹੋਣ ਵਾਲੇ ਖਤਰੇ ਬਾਰੇ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ ਅਤੇ ਮੈਨੂ ਇਹ ਯਕੀਨ ਹੈ ਕੇ ਇਹ ਖਤਰਾ ਬਹੁਤ ਮਾਮੂਲੀ ਹੈ। ਮੈਨੂ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਦਾ ਢੰਗ ਤਰੀਕਾ ਸਮਝਾ ਦਿੱਤਾ ਗਿਆ ਹੈ। ਮੈ ਇਸ ਖੋਜ ਕੰਮ ਵਿਚ ਹਿੱਸਾ ਲੈਣ ਬਾਰੇ ਆਪਣੀ ਰਜ਼ਾਮੰਦੀ ਕਿਸੇ ਵੀ ਸਮੇ ਵਾਪਸ ਲੈ ਸਕਦਾ ਹਾਂ/ ਸਕਦੀ ਹਾਂ ਅਤੇ ਮੇਰੀ ਰਜ਼ਾਮੰਦੀ ਵਾਪਸ ਲੈਣ ਦਾ ਮੇਰੇ ਇਲਾਜ ਤੇ ਕੋਈ ਅਸਰ ਨਹੀ ਹੋਵੇਗਾ।

_____ _____

ਗਵਾਹ ਦੇ ਹਸਤਾਖਰ ਮਰੀਜ਼ ਦੇ ਹਸਤਾਖਰ

ਮਿਤੀ_____ ਮਿਤੀ_____

ਪਤਾ _____ ਪਤਾ _____

_____

ਨਿਰੀਖਕ ਦੇ ਹਸਤਾਖਰ

_____

ਸਿਖਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ